The Isolation, Characterization and Chemical Properties of the Gibberellins[1]

by

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In the Orient, where the food supply is so precarious, rice diseases are a matter of grave concern; one of the most serious of these is the so-called "Bakanae" disease, which is caused by a fungus. It was first reported in Japan in 1898, and has since been observed in China, India, Ceylon, the Philippines, Italy, and most recently in various parts of Africa.

The Japanese call this condition the "foolish seedling" disease, because the plants grow unusually tall, and then die. In 1926, a Formosan phytopathologist by the name of Kurosawa became curious about the cause of this overgrowth of the rice plant, and decided to find out if some compound produced by the mold was responsible. He made the first step toward this goal by demonstrating that sterile culture filtrates of the fungus would, indeed, bring about the unusual hypertrophy characteristic of the disease. A number of attempts at isolation were made by Kurosawa and others, but many difficulties arose, and twelve years were to pass before the active principal could be obtained.

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[1] Presented August 28, 1956 at the Symposium on "Natural Plant Growth Regulators, other than Auxin" sponsored by the American Society of Plant Physiologists at the Storrs, Connecticut meeting of the American Institute of Biological Sciences.

During this time much effort was expended in an endeavor to unravel the tangled skein of facts and contradictions involved in the 'Bakanae' phenomenon. First, there was the problem of establishing the taxonomic position of the responsible organism, which was no easy task in a genus noted for morphological and physiological variation. The organism was considered by Hori in 1898 to be Fusarium heterosporum Nees. In 1917, the perfect stage of the organism was found by Sawada, who gave it the name of Lisea fujikuroi. Then in 1931, in the course of his extensive studies of the Fusaria, Wollenweber expressed the opinion that the fungus is identical with Fusarium moniliforme Sheldon, which is parasitic on corn, and accordingly he removed Lisea fujikuroi to the genus Gibberella as Gibberella fujikuroi (Sawada) Wollenw. It was found, however, that isolates from corn did not induce the 'Bakanae' phenomenon. It was concluded, therefore, that, although Wollenweber was justified in transferring the rice fungus to the genus Gibberella, there were still grounds for regarding the corn fungus Gibberella moniliformis (Sheld.) Wineland as distinct. These differences are slight and have never been regarded as sufficiently great for species separation, especially in the light of our more recent, fuller understanding of the variations in these organisms.

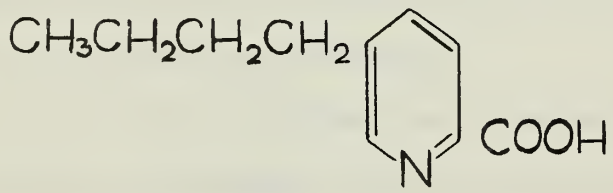
Besides the morphological vagaries, these organisms showed marked physiological variation; at times a culture would lose its pathogenicity completely on repeated transfer on synthetic media. Moreover, the ability to infect rice was not restored on return to the natural host.

Occasionally, too, a culture would bring about a stimulation of the growth of rice seedlings, and at other times a stunting of the growth.

Which of these two effects appeared seemed to depend on small changes in the cultivation temperature and the composition of the medium.

In view of all these difficulties, it is not surprising that progress was slow, but gradually the nature of the problem became more clearly defined. By the early 1930's, the sporadic attempts of isolated workers had been replaced by a concerted attack by a group of plant pathologists, plant physiologists, mycologists, biochemists, and organic chemists at the University of Tokyo.

By 1934, these workers (J. Agr. Chem. Soc. Japan 10, 1059-68 (1934)) had succeeded in isolating in pure crystalline form the growth-retarding factor, fusarinic acid, which proved to be 5-n-butyl picolinic acid. Its formula is shown on the first slide.



Fusarinic Acid

(Yabuta-University of Tokyo-1934)

Four more years of intensive effort followed, and then came the announcement of the isolation of the long sought growth-stimulating substance, to which the name "gibberellin A" was given (J. Agr. Chem. Soc. Japan 14, 1526 (1938)). It turned out to be a colorless, crystalline, optically active acid, and its potency was striking; one part in a million was sufficient to stimulate appreciably the growth of rice, wheat, barley, cucumbers and tobacco.

By the time of Pearl Harbor, seven papers on gibberellin had been published in Japanese by the University of Tokyo workers; by 1943 all but one of these papers had appeared in abstract form in this country, but apparently they aroused little interest among plant workers.

During the war the Japanese published twelve more gibberellin papers in their journals; unfortunately, these were not abstracted in this country until 1950, which prolonged the neglect of the subject. In that same year, the first work on gibberellin outside of Japan was described at a phytopathology meeting by Mitchell and Angel of the U. S. Army Chemical Corps Biological Laboratories at Camp Detrick in Maryland. Much work had been done at this laboratory, during and after the war, on compounds having effects on plants, so the studies on gibberellin were a natural outgrowth of that interest. All attempts by Mitchell and Angel to prepare crystalline gibberellin, however, had failed, although gummy concentrates with activity had been obtained.

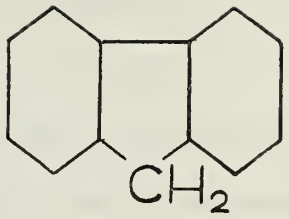
In August, 1951, arrangements were made with our laboratory to develop a reliable fermentation process for the production of gibberellin, and to work out a procedure for its complete purification. A study of the five Japanese papers on the chemistry of gibberellin gave us the facts listed on the next slide; the work is that of Sumiki and his students at the University of Tokyo.

Properties of Gibberellin A (1950)

(Sumiki - University of Tokyo)

1. White crystals, difficulty soluble in water.
2. Molecular formula $C_{22}H_{26}O_7$.

3. M.P. 242° C.
4. $[\alpha]_D$ +36°.
5. An acid, as indicated by ester formation.
6. Shown by degradation studies to have a fluorene nucleus, as the fundamental ring system.



Our first efforts to duplicate the Japanese work failed, even though we were using a sub-culture of their organism and their cultural conditions insofar as we could duplicate them. However, by using a different strain of the organism (supplied by Dr. J. E. Mitchell of Camp Detrick) and other fermentation conditions, Dr. Raper and Miss Fennell were able to obtain good production of gibberellin, and soon means were devised for the isolation of crude crystalline material. The large-scale fermentations were carried out in 300-gallon aerated tanks; the yields were quite reproducible, and averaged about 12 grams of crystals per 160 gallons of culture liquor. This work is described in a paper entitled "The Microbiological Production of Gibberellins A and X" (Stodola, Raper, Fennell, Conway, Sohns, Langford and Jackson, Archives of Biochemistry and Biophysics 54, 240-245 (1955)).

Our crystalline product had a rotation of about +65°, and hence was obviously different from the gibberellin A of the Japanese, which was reported to have a rotation of +36°. It soon became apparent that our product was a mixture of very similar compounds which could not be separated by crystallization. After considerable effort we were able to develop a chromatographic method by means of which we could separate

the mixture into a compound that appeared to be the same as the Japanese gibberellin A, and a new gibberellin with a rotation of +91°, which we tentatively designated as gibberellin X. (Our first announcement of the new gibberellin was in the 1953 Report of the Chief of the Bureau of Agricultural and Industrial Chemistry, U.S.D.A., page 74.) The efficiency and reproducibility of our chromatographic method are illustrated by the curves shown on the next slide.

See attached figure.

A paper giving the details of this work has been submitted for publication in the Archives of Biochemistry and Biophysics (The Separation of Gibberellin A and Gibberellic Acid on Buffered Partition Columns by Stodola, Nelson and Spence).

To characterize our two gibberellins we carefully established their molecular formulas, and found that they differed by only two hydrogens. Moreover, our formula for gibberellin A was quite different from that given by Sumiki. Our formulas are shown on the next slide.

Molecular Formulas of N.U.R.B. Gibberellins (1954)

Gibberellin A	Gibberellin X
$C_{19}H_{24}O_6$	$C_{19}H_{22}O_6$
(one double bond)	(two double bonds)
$[\alpha]_D^{25}$ + 36°	$[\alpha]_D^{25}$ + 91°
(Sumiki formula $C_{22}H_{26}O_7$)	

Because of the disparity between our formula for gibberellin A and that of the Japanese, I wrote to Sumiki on April 7, 1954, and told him of our findings. In the light of our results, he decided to re-examine his gibberellin A for purity. He found, by means of column chromatography, that his so-called "gibberellin A" actually was a mixture of three gibberellins, two of which were the same as the ones we had isolated. (Isolation of Gibberellins and their Properties by Takahashi, Kitamura, Kawarada, Seta, Takai, Tamura and Sumiki, Bull. Agri. Chem. Soc. Japan 19, 267-277 (1955)). The formulas of Sumiki's gibberellins are given on the next slide.

Molecular Formulas of Japanese Gibberellins (1955)

A_1	A_2	A_3
$C_{19}H_{24}O_6$	$C_{19}H_{26}O_6$	$C_{19}H_{22}O_6$
$[\alpha]_D$ + 42.3°	$[\alpha]_D$ + 11.7°	(obtained only as methyl ester)
[Same formula as N.U.R.B. gibberellin A]		[Same formula as that of N.U.R.B. gibberellin X]

Just as we finished our work, there appeared a paper by Curtis and Cross (Chem. and Industry 1954, 1066) of Imperial Chemical Industries in England which described the isolation of a new gibberellin that appeared to be the same as our gibberellin X. We exchanged samples with the British workers, and it was soon established that the compounds are identical. Curtis and Cross called their product gibberellic acid, and we have now adopted that name.

At this point it might be advisable to list the members of the gibberellin family so we will be sure to have their names, formulas and properties correct. These are shown on the next slide.

The Gibberellins

$C_{19}H_{24}O_6$	Gibberellin A_1 (Sumiki: M.P. 232-5°; $[\alpha]_D$ + 42.3°)
	Gibberellin A (N.U.R.B.: M.P. 255-8°; $[\alpha]_D$ + 36°)
$C_{19}H_{26}O_6$	Gibberellin A_2 (Sumiki: M.P. 235-7°; $[\alpha]_D$ + 11.7°)
$C_{19}H_{22}O_6$	Gibberellin A_3 (Sumiki: Obtained only as methyl ester)
	Gibberellin X (N.U.R.B.: M.P. 232-5°; $[\alpha]_D$ + 91°)
	Gibberellic Acid (I.C.I.: M.P. 233-5°; $[\alpha]_D$ + 83°)

These are the three natural gibberellins; Sumiki has compared their activities using a rice seedling assay, and found all of them to have about the same potency.

Now I would like to say something about the structure of gibberellic acid, which is being actively investigated by the I.C.I. workers. They have already published three papers (Cross, J. Chem. Soc., 1954, 4670-6; Mulholland and Ward, ibid. 1954, 4676-81, 2415-17) on the subject, and have another one in press. The next slide will show their work on the degradation of gibberellic acid.

British Work

on

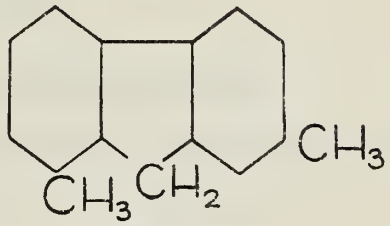
Degradation of Gibberellic Acid

$C_{19}H_{22}O_6$ —(dilute acid, at 50°)→ Allogibberic Acid + CO_2 + H_2O —(dilute acid, at 100°)→

Allogibberic Acid
$C_{18}H_{20}O_3$
M.P. 200° $[\alpha]_D$ - 80°

Gibberic Acid
$C_{18}H_{20}O_3$
M.P. 150° $[\alpha]_D$ + 7°

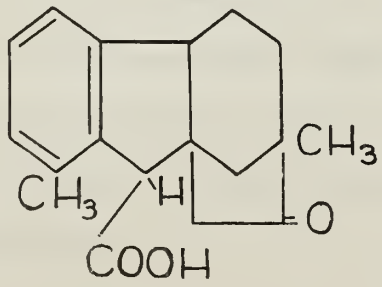
—(Se, Δ)→



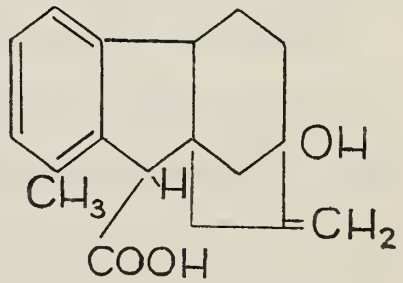
Gibberene

(1,7 - dimethyl fluorene)

Last week I received from Dr. Grove, who is in charge of the structure work at I.C.I., an advance copy of the paper now in press. (Gibberellic acid. Part IV. The Structures of Gibberic and Allogibberic Acid and Possible Structures for Gibberellic Acid by Cross, Grove, MacMillan and Mulholland. To be published in Chemistry and Industry.) In the paper are given the following formulas for gibberic and allogibberic acid.

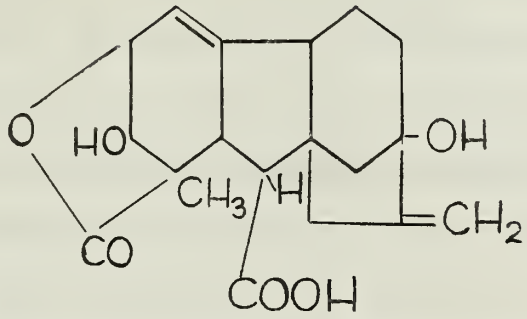


Gibberic Acid
$C_{18}H_{20}O_3$



Allogibberic Acid
$C_{18}H_{20}O_3$

On the basis of their degradation studies, the British have arrived at the tentative formula for gibberellic acid which you see on the next slide.



Tentative Structure for Gibberellic Acid

The points of attachment of the lactone group to the ring are still in doubt.

From this structure, it can be seen that the gibberellins are unrelated chemically to any of the known plant growth regulators.

As plant physiologists, you will probably be interested in the availability of the gibberellins for testing purposes. In our work we prepared about 50 grams of crude crystalline gibberellin, of which a considerable portion is still left. We also prepared by chromatography about a gram each of pure gibberellin A and pure gibberellic acid, and small amounts of these remain. So far, I have sent out almost a hundred samples to about fifty investigators, and as long as our supplies last we shall be glad to provide 100-mg. samples of the mixture to anyone who wishes to study its effects on plants; if anything of interest should turn up, we would be able to supply small amounts of the pure gibberellins for crucial experiments.

To find out about the British supplies of gibberellic acid, I wrote recently to Dr. P. W. Brian, who is in charge of the gibberellin work at Imperial Chemical Industries (Akers Research Laboratories, The Frythe, Welwyn, Herts, England). He stated that he is 'prepared to supply for research purposes samples up to 100 mg. free of charge to a limited number of academic or other non-industrial workers.' He further stated that they are still making the material on a laboratory scale only, and hence applications for larger amounts would have to be considered on their merits, and in such cases, if they could supply the material, they would probably have to charge for it.

In view of our supplies and those of the British, I think it is safe to say that there is adequate material for laboratory testing on a very wide scale. That brings up a final point: What are the prospects for large-scale manufacture in case some agricultural use should be found?

From our pilot-plant work in 300-gallon fermentors (160 gallons of culture liquor) we could conclude that commercial production would present no problem. Even though we made no attempt to get maximum yields, we obtained, in a 3-day fermentation, 12 grams of crystalline product from 160 gallons of culture liquor. Working at about 1/8th our scale, the British workers (Borrow et al. J. Sci. Food & Agr. 6, 340-48 (1955)) found that their yield of gibberellin increased steadily up to the 18th day when it amounted to about nine times what we obtained in 3 days. There seems to be little doubt about our ability to make plenty of gibberellin, although it is apt to be a rather expensive chemical for awhile. A number of pharmaceutical companies in this country are already producing gibberellin on a pilot-plant scale, so we can look forward to improvements soon, both in yields and methods of isolation.

Our culture of Fusarium moniliforme is available upon request to anyone interested in the preparation of gibberellin; we also have for distribution five of the best British cultures (numbers 917, 1001, 1004, 1135 and 1139), as well as the Japanese strain.

In conclusion I would like to say that I have found the gibberellin problem to be a very interesting one; it has had somewhat more than the usual quota of errors, confusions and frustrations, but fortunately these have been well counterbalanced by some good breaks, some pleasant surprises, and a lot of satisfaction. I feel that we are all greatly in debt to the Japanese for having placed in our hands such fascinating compounds; that the plant physiologist has been making the most of this opportunity will, I am sure, be amply demonstrated by our next speaker.

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July 1958

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
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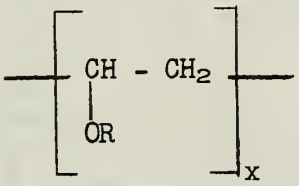
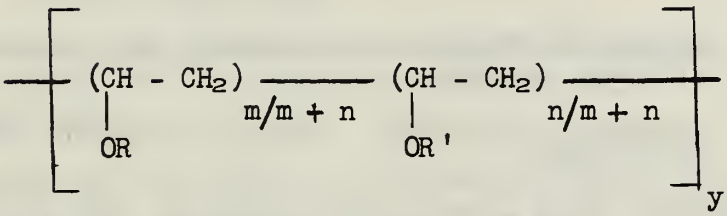
INFORMATION ON POLYMERS AND COPOLYMERS OF VINYL ETHERS DERIVED FROM LINSEED AND SOYBEAN OILS

Structure

Homopolymers:



Copolymers:



R = Polyunsaturated fatty radical derived from linseed or soybean alcohols

R' = Lower alkyl radical (ethyl, isobutyl, allyl, etc.)

m = Moles of fatty vinyl ether in copolymer

n = Moles of lower alkyl vinyl ether in copolymer (arrangement in chain probably random)

x = Units in polymer chain

y = Units in copolymer chain

Types of Products

Homopolymers can be prepared from linseed or soybean vinyl ethers in which the fatty unsaturation is either conjugated or nonconjugated. Copolymers can be prepared from either conjugated or nonconjugated linseed or soybean vinyl ethers with various proportions of lower alkyl vinyl ethers. Typical comonomers include ethyl, isobutyl, allyl, 2-chloroethyl, and 2-ethylhexyl vinyl ethers, etc.

Properties of Products

Depending upon choice of monomers, polymers or copolymers having differing properties can be obtained. Properties of two typical products are as follows:

	3:1[a] Isobutyl-conjugated soybean copolymer	Nonconjugated linseed homopolymers
Molecular weight	5,000	4,700
Viscosity (Gardner)	>Z-6	Z-1
Color (Gardner)	6	3
Iodine value	53	185
Conjugation, %		
Diene	18.7	1.4
Triene	0.2	0.11
Refractive index (n_D^{30})	1.4660	1.4845
Specific gravity	0.926	0.907
Film formation	Baked (150°-225° C.)	Air-dry

Solubility: Soluble in aromatic and aliphatic hydrocarbons, halogenated solvents, pyridine

Insoluble in alcohols, acetone, formamide, "Cellosolve," "Carbitol"[b]

Compatibility with other resins or film-formers: Not yet determined.

[a] Molar ratio of comonomers.

[b] Mention of trade names is for information only and does not constitute an endorsement by the U. S. Department of Agriculture

Film Formation

Excellent films are obtained from polymers and copolymers containing conjugated soybean or linseed vinyl ether by baking. Polymers of nonconjugated linseed vinyl ether and copolymers containing up to 50-mole percent of nonfatty comonomer show pronounced air-drying properties and may be used for either air-drying or baking finishes.

Optimum conditions for film formation from the various polymers and copolymers are yet to be established. The following information will serve as a guide for experimentation.

Many of the conjugated soybean polymers and copolymers will produce films sufficiently hard for some purposes by baking for 10 minutes at 450° F. without added driers. Small amounts of conventional driers can be added to obtain hard films at lower temperatures. Excessive amounts of driers may cause oxidative degradation with loss of desirable film properties.

Film formation appears to be influenced to some extent by the nature of the substrate. Thus, films on black iron, "Hinac," or aluminum frequently are superior to films produced under the same conditions on electrolytic or hot-dipped tin plate.

Air-drying polymers and copolymers form films with normal amounts of drier. Thus, the nonconjugated linseed homopolymer with 0.6 percent of lead dries to a hard film in 1 to 1.25 hours.

Film Properties

Properties have been studied for films prepared from drier and polymer or copolymer. No information is presently available on pigmented films or films from formulations containing other resins or film-forming ingredients. Films were coated on metal or glass.

Hardness: The hardness of baked films varies from 4 to 42 (Sward) depending upon the nature of the polymer or copolymer used, the drier, and/or the baking cycle. Air-dried linseed homopolymer films attain a hardness of 8.

Color: The color of baked films varies from light-yellow to amber. Discoloration on baking is less marked than is observed with many other fat-derived film-formers.

Chemical Resistance: Resistance of the dried films is excellent to outstanding to water, 18 percent hydrochloric acid, 5 percent sodium hydroxide, and to such solvents as alcohols, mineral spirits, mineral oil, hexane, and benzene. Some solvents, such as acetone or chloroform, may cause swelling of the film. A typical baked film from a 3:1 isobutyl-conjugated soybean vinyl ether copolymer resisted 5 percent sodium hydroxide for 24 hours and showed slight swelling after 7 hours' immersion in chloroform.

Adhesion: Baked films show exceptionally good adhesion to metals, particularly black iron, "Hinac," and aluminum. Coated stock may be fabricated, for example, into a can end, without damage to the film. Abrasion resistance is excellent.

Publications

Further information on these polymers and copolymers may be found in the following publications:

Teeter, H. M. Dufek, E. J., Coleman, C. B., Glass, C. A., Melvin, E. H., and Cowan, J. C. 1956 Reaction of Unsaturated Fatty Alcohols. I. Preparation and Properties of Some Vinyl Ethers. J. Am. Oil Chemists' Soc., 33: 399-404.

Schneider, W. J., Gast, L. E., Melvin, E. H., Glass, C. A., and Teeter, H. M. 1957 Reaction of Unsaturated Fatty Alcohols. II. Polymerization of Vinyl Ethers and Film Properties of Polymers. J. Am. Oil Chemists' Soc., 34: 244-247.

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Gast, L. E., Coleman, C. B., and Teeter, H. M. Reaction of Unsaturated Fatty Alcohols. IV. Oxidative Degradation of Lauryl Isopropyl Ether. J. Org. Chem. (in press).

Gast, L. E., Schneider, W. J., O'Donnell, J. L., Cowan, J. C., and Teeter, H. M. 1958 Reaction of Unsaturated Fatty Alcohols. V. Preparation and Properties of Some Copolymers of Unsaturated Fatty Vinyl Ethers with Lower Alkyl Vinyl Ethers. J. Am. Oil Chemists' Soc. 35: 347-350.

Gast, L. E., Teeter, H. M., and Cowan, J. C. 1958 Vinyl Ethers of Polyunsaturated Fatty Alcohols: Promising New Materials for Protective Coatings. Preprints of papers presented at San Francisco Meeting, Division of Paint, Plastics and Printing Ink Chemistry, American Chemical Society, pages 160-169.

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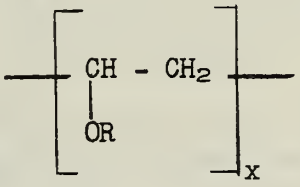

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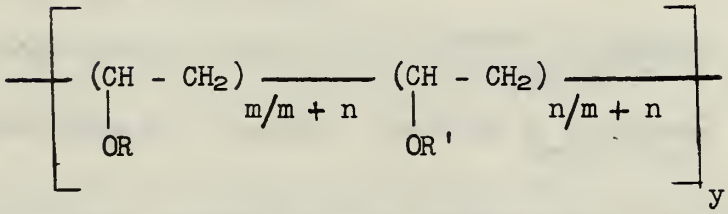

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Hardness: The hardness of baked films varies from 4 to 42 (Sward) depending upon the nature of the polymer or copolymer used, the drier, and/or the baking cycle. Air-dried linseed homopolymer films attain a hardness of 8.

Color: The color of baked films varies from light-yellow to amber. Discoloration on baking is less marked than is observed with many other fat-derived film-formers.

Chemical Resistance: Resistance of the dried films is excellent to outstanding to water, 18 percent hydrochloric acid, 5 percent sodium hydroxide, and to such solvents as alcohols, mineral spirits, mineral oil, hexane, and benzene. Some solvents, such as acetone or chloroform, may cause swelling of the film. A typical baked film from a 3:1 isobutyl-conjugated soybean vinyl ether copolymer resisted 5 percent sodium hydroxide for 24 hours and showed slight swelling after 7 hours' immersion in chloroform.

Adhesion: Baked films show exceptionally good adhesion to metals, particularly black iron, "Hinac," and aluminum. Coated stock may be fabricated, for example, into a can end, without damage to the film. Abrasion resistance is excellent.

Publications

Further information on these polymers and copolymers may be found in the following publications:

Teeter, H. M. Dufek, E. J., Coleman, C. B., Glass, C. A., Melvin, E. H., and Cowan, J. C. 1956 Reaction of Unsaturated Fatty Alcohols. I. Preparation and Properties of Some Vinyl Ethers. J. Am. Oil Chemists' Soc., 33: 399-404.

Schneider, W. J., Gast, L. E., Melvin, E. H., Glass, C. A., and Teeter, H. M. 1957 Reaction of Unsaturated Fatty Alcohols. II. Polymerization of Vinyl Ethers and Film Properties of Polymers. J. Am. Oil Chemists' Soc., 34: 244-247.

Gast, L. E., Schneider, W. J., and Teeter, H. M. 1957 Reaction of Unsaturated Fatty Alcohols. III. Viscosity and Molecular Weight Studies on Some Vinyl Ether Polymers. J. Am. Oil Chemists' Soc., 34: 307-310.

Gast, L. E., Coleman, C. B., and Teeter, H. M. Reaction of Unsaturated Fatty Alcohols. IV. Oxidative Degradation of Lauryl Isopropyl Ether. J. Org. Chem. (in press).

Gast, L. E., Schneider, W. J., O'Donnell, J. L., Cowan, J. C., and Teeter, H. M. 1958 Reaction of Unsaturated Fatty Alcohols. V. Preparation and Properties of Some Copolymers of Unsaturated Fatty Vinyl Ethers with Lower Alkyl Vinyl Ethers. J. Am. Oil Chemists' Soc. 35: 347-350.

Gast, L. E., Teeter, H. M., and Cowan, J. C. 1958 Vinyl Ethers of Polyunsaturated Fatty Alcohols: Promising New Materials for Protective Coatings. Preprints of papers presented at San Francisco Meeting, Division of Paint, Plastics and Printing Ink Chemistry, American Chemical Society, pages 160-169.

CA-N-7
October 1958

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

INFORMATION ON PHOSPHOMANNAN Y-2448

This product is the result of the research efforts of a team of microbiologists, carbohydrate chemists, and chemical engineers. It represents one of a series of polysaccharides to be made by fermentative processes.

Nature and Origin: A high molecular weight, exocellular polysaccharide from the yeast, Hansenula holstii NRRL Y-2448. The molecular homogeneity is high and the molecular weight of the order of millions. For convenience, the product is designated as Phosphomannan Y-2448.

Preparation: Whole culture fermentation of a medium containing 6 percent commercial glucose, organic nitrogen sources, potassium dihydrogen phosphate and trace elements. Incubation time, 96 hours, at 28° C., aerobic conditions.

Purification and Isolation: Supercentrifugation to remove cells, precipitation by methanol in the presence of electrolyte such as potassium chloride, reprecipitations, and finally dehydration by adding aqueous solution to methanol. The yield is 37 percent based on glucose; this should be increased by further research.

Composition and Structure: Repeating unit consists of 5 anhydromannose units one of which carries a monopotassium ortho phosphate group attached at C_6 position. All phosphate groups appear to be in diester form through cross-linking of chains.

Properties:

Gum.--Long, cohesive, semi-firm, adheres to dry surfaces.

Solid.--White powder, readily soluble in cold water. Absorbs 13.5 percent moisture at 20° C. and 50 percent relative humidity. Contains essentially no free inorganic salt.

Solution.--Clear, thixotropic, with gel-like aspect at concentrations greater than about 1 percent. pH in range 5.5 to 7.0 for concentrations of about 0.2 to 5.0 percent, respectively.

Viscosity: Viscosity-concentration curves of Phosphomannan Y-2448 in water, in potassium chloride solution, and in borax solution are shown in Figures 1, 2, and 3, respectively. Increase of viscosity of the polysaccharide solutions by borax is accompanied by progressive increase in ropiness and cohesiveness, and finally leads to gel formation. All viscosity data were obtained at 25° C. with a Brookfield viscometer operating at 30 rpm.*

Solutions containing polysaccharide in concentrations about 1.5 percent or greater with added borax, show still further increase in viscosity upon addition of potassium chloride. However, on a weight basis, potassium chloride is less efficient in increasing viscosity than would be further addition of borax.

Acid Form of Polysaccharide: May be formed from the potassium salt by use of cation exchange resin, or by dialysis of a solution acidified with acetic acid. Solutions of the acid form have pH near 3.0 for concentrations of about 0.2 to 2.0 percent.

Suggested Uses: Phosphomannan Y-2448 is expected to find practical applications based on its colloidal properties, its formation of complexes and of salts with inorganic- and organic-type bases, and its esterification through the phosphate group.

Further information on preparation and properties of this polysaccharide will be sent on request.

* This equipment is named merely as part of the exact experimental conditions. Naming it does not constitute an indorsement of this apparatus over those of other manufacturers.

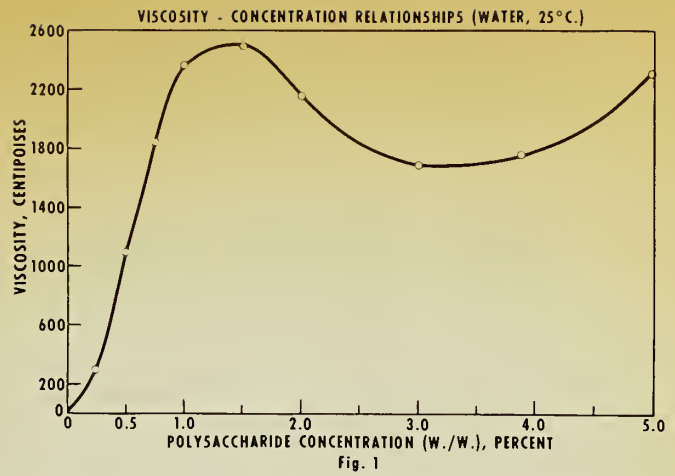

Fig. 1

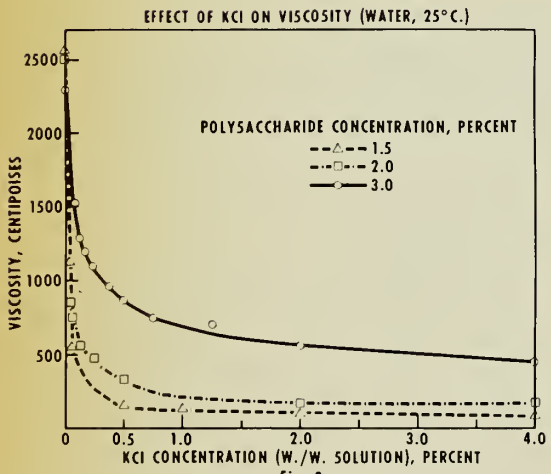

Fig. 2

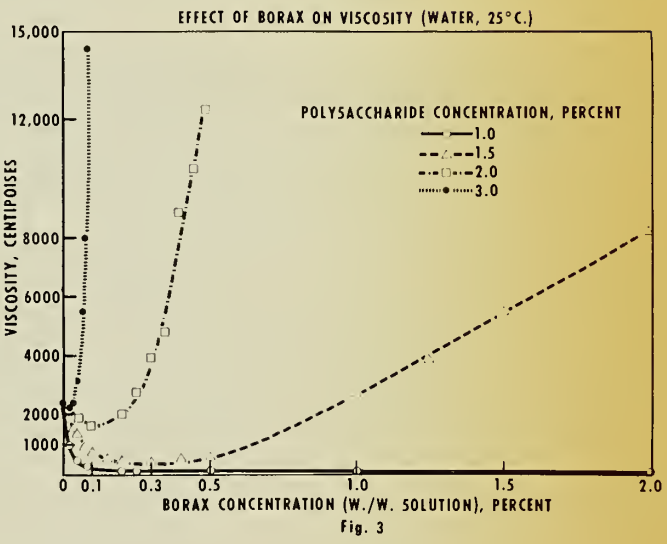

Fig. 3

CA-N-8
October 1958

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

DATA SHEET FOR DICARBOXYL STARCHES*

Dicarboxyl starches are new starch derivatives containing sodium dicarboxylate units. The dicarboxyl starches [J. AMER. CHEM. SOC., 79, 6457 (1957)] are obtained by chlorous acid oxidation of dialdehyde starches. The range of oxidation is from 0.5 to 5.0% (number of dicarboxyl units per 100 repeating units). Essentially, all dialdehyde groups are oxidized to dicarboxylate groups.

In addition to the variation of oxidation level, parent starches may be cross-linked to varying extents prior to oxidation which will give a number of different products for a given dicarboxyl content. The cross-linking (epichlorohydrin) is expressed as anhydroglucose units/cross-link and varies from 300 to 2,000. The laboratory preparations available represent several combinations of cross-linking and oxidation level.

GENERAL PHYSICAL PROPERTIES

Appearance is similar to unmodified starch because it retains a granular form.

Equilibrium moisture content is 15 - 16% at 21° C. and 65% relative humidity.

pH of 2% distilled water slurry is 8.4 - 9.0

pH of 2% cooked paste is 6.0 - 7.0.

Clarity of 1% cooked pastes is 81 - 99% transmission compared to 64% for unmodified starch.

Stability on storage at 15% moisture content and room temperature is excellent.

Viscosity stability of 2% cooked pastes at 25° C. is very good and no gelling occurs on cooling.

METHOD OF DISPERSION

Aqueous dispersions or pastes of the dicarboxyl starches are obtained readily by heating mechanically stirred slurries. Choice of slurry concentration is dictated by the viscosity requirements in the final product. Each dicarboxyl starch upon heating reaches a temperature at which swelling occurs rather sharply with an accompanying viscosity rise. The final products are short, smooth, translucent pastes. Broad ranges of viscosity, viscosity stability, and ease of dispersion are obtainable depending upon the dicarboxyl starch employed. For each degree of oxidation there is a level of cross-linking that will give an optimum balance of viscosity and viscosity stability.

*These data are preliminary and are provided to aid in evaluation of the sample they accompany. As later information develops, it will be available on request.

The Corn Industries Research Foundation viscometer (CIRF),** has been used extensively to investigate the flow characteristics of these products at various stages in the cooking process. The paste temperature reached in the CIRF viscometer after the first 18 minutes is about 90° C. Cooking curves obtained with various dicarboxyl starches in the CIRF viscometer are shown in Figure 1.

The cold-paste Brookfield** viscosity (20 r.p.m. - 25° C.) for a given dicarboxyl starch is dependent upon the time-temperature cooking history. As an example, at 2% concentration, three dicarboxyl starches had viscosities ranging from 5,000 to 13,000 centipoises.

FACTORS INFLUENCING DISPERSION AND VISCOSITY CHARACTERISTICS

The paste viscosities of the dicarboxyl starches are affected by several factors:

1. **Concentration** - Dicarboxyl starches are similar to most natural and synthetic gums in that small increases in concentration result in large viscosity increases.

2. **Presence of electrolytes** - Salts cause large decreases in viscosity which are reversible on removal of the electrolyte.

3. **pH** - Cold paste viscosity is relatively stable over pH range 6-9.

4. **Temperature** - Cooking at temperatures lower than 92° C. results in slightly longer times for gelatinization, lower maximum viscosities, and increased viscosity stability.

**This equipment is named merely as part of the exact experimental conditions. Naming it does not constitute an endorsement of this apparatus over those of other manufacturers.

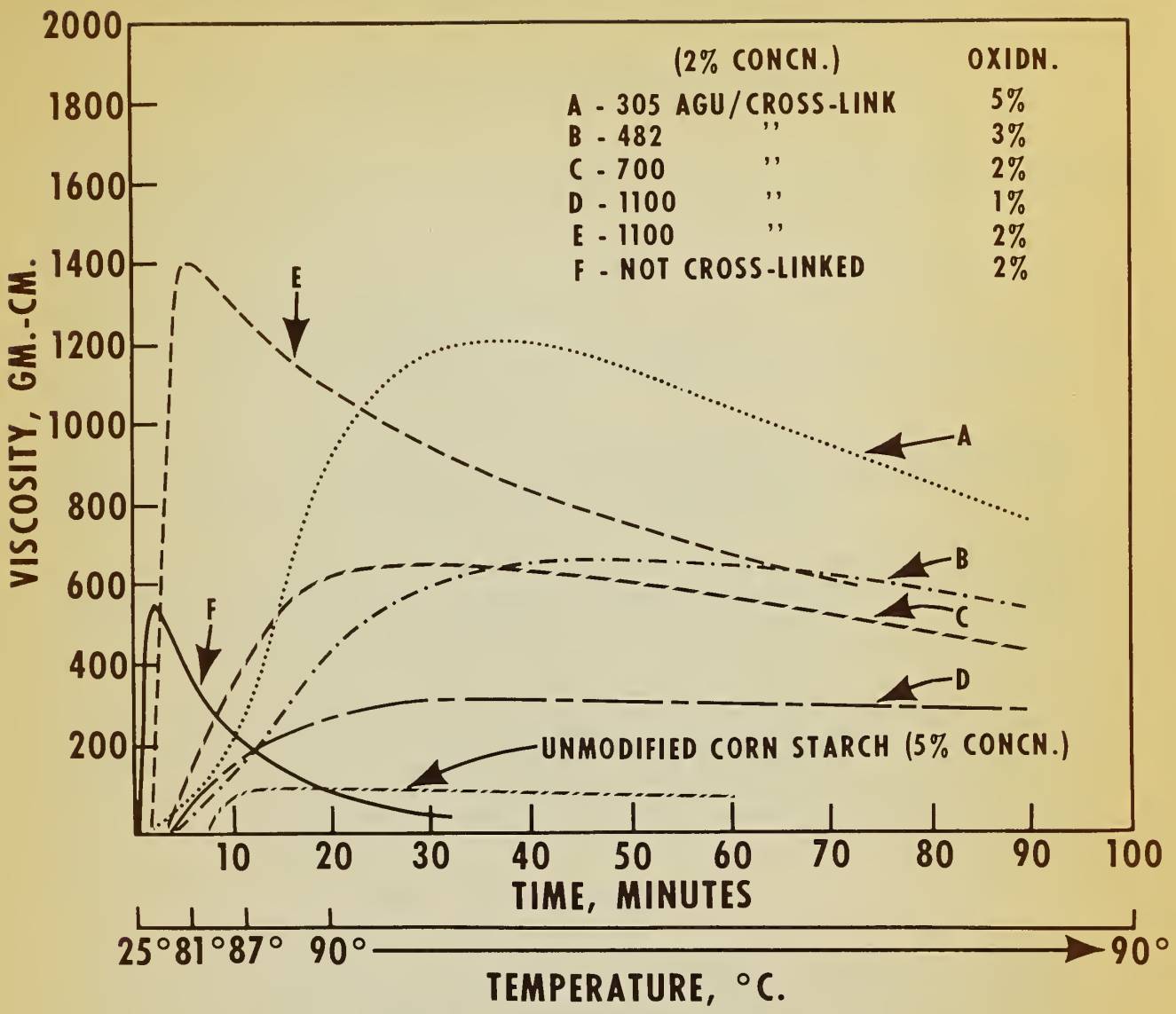

Fig. 1. Viscosity behavior of dicarboxyl starches at 2% concentration on heating as measured in the CIRF viscometer. For comparison the pasting curve is given for unmodified corn starch at 5% concentration.

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September 1959

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

INFORMATION ON POLYSACCHARIDE B-1459

This product is the result of the research efforts of a team of microbiologists, carbohydrate chemists, and chemical engineers. It is one of a series of polysaccharides to be made by fermentation processes.

Research on this polysaccharide is still in progress; a number of problems on production and properties have not yet been investigated.

Nature and Origin: A high molecular weight, exocellular, heteropolysaccharide from the bacterium, *Xanthomonas campestris* NRRL B-1459.

Preparation: Whole culture fermentation of a medium containing 2-5 percent commercial glucose, organic nitrogen sources, dipotassium hydrogen phosphate, and appropriate trace elements. Incubation time 96 hours, at 28° C., aerobic conditions.

Purification and Isolation: Centrifugation to remove cells, precipitation by methanol in the presence of electrolyte such as potassium chloride, reprecipitations, and finally dehydration of the fibrous gum by methanol. Other methods of purification are under investigation. Yields up to 50 percent based on glucose are being obtained currently.

Composition: Polysaccharide B-1459 contains mannose, glucose, potassium glucuronate, and acetyl in the approximate molar ratio of 2:1:1:1. The potassium content is about 5.4 percent of which 0.3 percent or less derives from potassium chloride. The approximately 0.4 percent nitrogen and 0.2 percent phosphorus present in current preparations are believed to be extraneous material which will be decreased through improved processing.

Properties:

Gum: Voluminous, noncohesive, somewhat fibrous.

Solid: As now prepared on a pilot-plant scale, the solid is a soft, bulky powder slightly colored by pigment from the culture. It swells and dissolves completely in cold water. When humidified and stored under conditions of 50 percent relative humidity at 20° C., the polysaccharide absorbs about 15 percent moisture and is stable for at least a year.

Aqueous Solutions: Opalescent, nonthixotropic; show weak, soft gelation. The pH is in range 7.0 to 8.5 for concentrations 0.1 to 1.0 percent. Addition of potassium chloride decreases the pH somewhat.

Specific Rotation: Essentially zero.

Film Formation: Preliminary experimentation on films plasticized with glycerol has given representative values of tensile strength 5 kg./mm.^{2}, elongation 16 percent, and double folds 3,500.

Viscosity Relationships:* Viscosity-concentration curves of Polysaccharide B-1459 in water and in representative salt solutions are shown in Figures 1, 2, and 3. Increase in viscosity in the presence of salt (Figure 2) is anomalous for a polyelectrolyte and thus is a property not shared by many of the other hydrophilic colloids now in use.

The effect of heating in presence or absence of a salt such as potassium chloride is shown in Figure 4. For polysaccharide concentrations $\geq$0.5 percent, potassium chloride in the solution moderates or eliminates the decrease in viscosity caused by heat. Polysaccharide reisolated after heating to about 90° C. shows the characteristic increase in viscosity upon addition of salt and no evidence of molecular degradation (Figure 1). Stability of viscosity to heat in the presence of salt differentiates this polysaccharide from most hydrophilic colloids now in use.

Deacetylated Polysaccharide B-1459: Is obtained by short treatment with dilute alkali at room temperature with exclusion of oxygen. The product shows the characteristic increase in viscosity upon addition of salt and no indication of molecular degradation (Figure 1). This product might be found to have some advantages over the unmodified polysaccharide in possible applications.

Applicability: The properties of the dilute solutions of possible significance for applications are the anomalous increase in viscosities caused by salts and the stability of viscosities toward heat, especially when salt is present.

The polysaccharide is of interest because of three properties which may have potential for future applications. These are (a) the easy preparation from aqueous solutions of films which have high flexibility; (b) the ready loss of acetyl upon mild hydrolysis to give an unsubstituted polysaccharide having enhanced properties of value; and (c) although pharmacological tests have not been conducted thus far, from the nature of the constituent sugars one would not expect incompatibility to animal organisms.

Specific details on production, purification, and handling can be made available to interested parties.

* All viscosity data were obtained at 25° C. with a Brookfield viscometer operating at 30 r.p.m. This equipment is named merely as part of the exact experimental conditions. Naming it does not constitute an endorsement of this apparatus over those of other manufacturers.

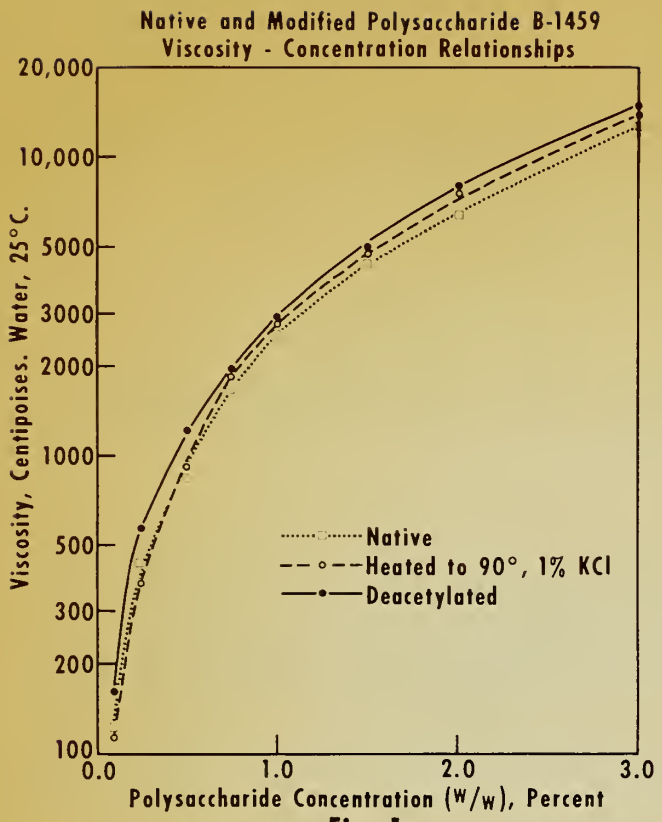


Fig. 1

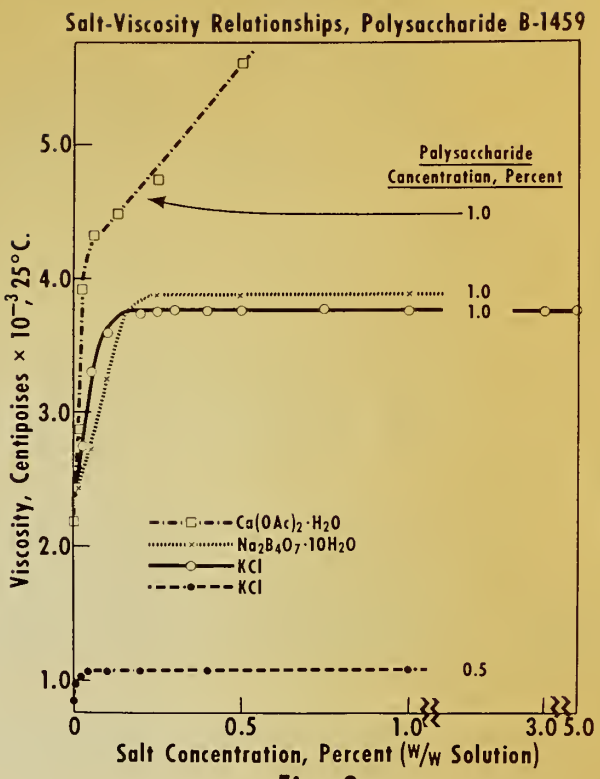


Fig. 2

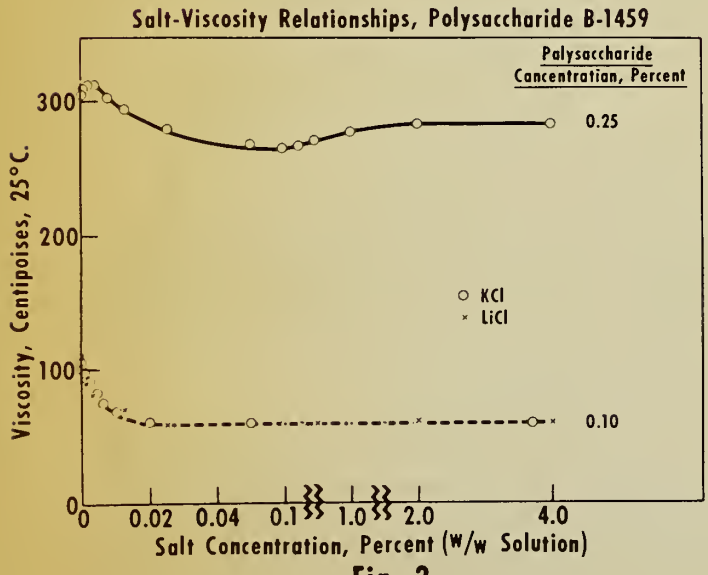


Fig. 3

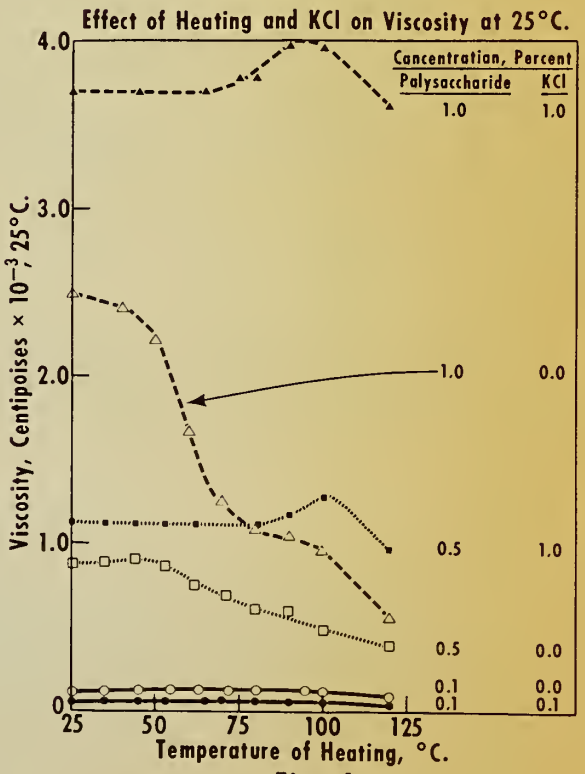


Fig. 4

CA-N-10
April 1960

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

DIALDEHYDE STARCH

Use as Additive for Wet Strength Paper

Dialdehyde starch, a polymeric dialdehyde developed by the U.S. Department of Agriculture, imparts wet strength to paper products either by wet end addition (1) or by surface application (2). Other properties such as dry tensile strength, burst strength, and fold endurance are also improved by application of this dialdehyde.

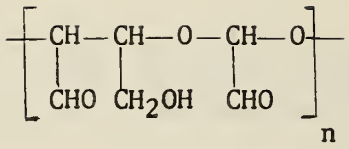
Desirable features associated with the use of dialdehyde starch for wet strength paper are:

1. Rapid development of wet strength in the sheet with no extended cure or additional heating required.
2. Simplicity of preparing aqueous dispersions of dialdehyde starch.
3. Substantial improvement in dry tensile and burst strengths and increased fold endurance.
4. Ease of broke recovery.
5. Storage stability of dialdehyde starch.

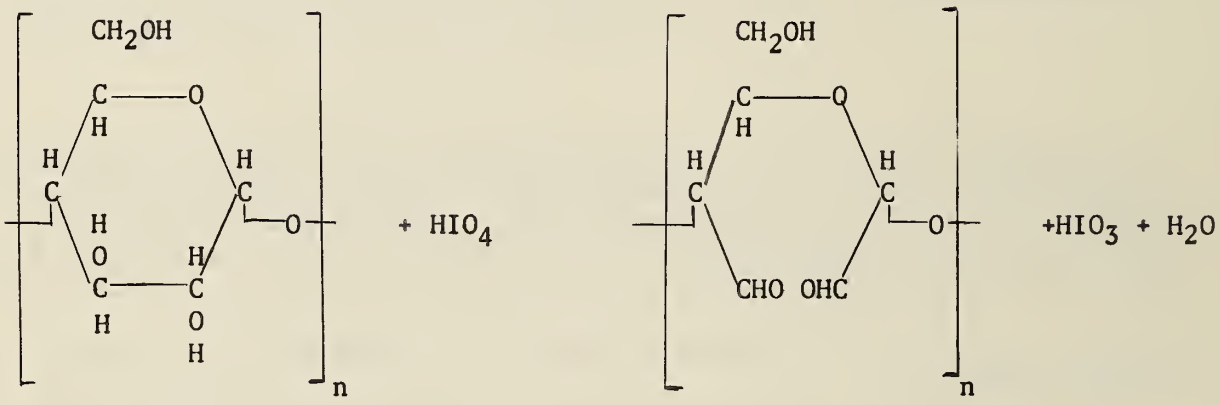
The following description of the nature and properties of dialdehyde starch and the experimental conditions developed for its use in improving the wet strength of paper are provided as a guide to those wishing to make trials of dialdehyde starch in this application.

Properties of Dialdehyde Starch

Commercial grade dialdehyde starch (3,4) is a finely divided, practically odorless solid very similar in appearance and color to the parent starch. Although it retains the granule form of the starch from which it is prepared, dialdehyde starch possesses completely different chemical properties. In fact, it is a polymeric dialdehyde whose monomeric units can be represented as:



Dialdehyde starch is produced by periodic acid oxidation of starch according to the equation:



The reactivity of the carbonyl function has suggested many possible uses for dialdehyde starch among which is its application in papermaking as a wet strengthening agent. For this purpose a product of essentially maximum oxidation, which contains at least 90 dialdehyde units per 100 units of the polymer, is more advantageous to use. Dialdehyde starch is applied most effectively in aqueous dispersions in which the granules are disrupted in such a manner that controlled depolymerization results. The appropriate molecular weight range of the polymeric dialdehydes produced is most important in wet end application. Wet strength values up to 30 percent of the dry strength of treated paper can readily be obtained with such dispersions by wet end addition. Wet strength of 50 percent is achieved by surface application where a greater quantity of the polymeric dialdehyde is absorbed.

Preparation of Aqueous Dispersion

Dialdehyde starch may vary in dispersion characteristics depending upon the method of preparation and upon conditions and length of storage of the dry product. The following procedure is recommended to obtain aqueous dispersions most effective for development of wet strength in paper by addition prior to web formation:

Thirty-three parts by weight of dialdehyde starch are added to 967 parts of water with continuous mechanical stirring and then 4.5 parts of sodium bisulfite are added. The slurry is heated rapidly with agitation to 90°-95° C. and is maintained at that temperature until the dialdehyde starch has passed through a thick paste stage. Heating and agitation are continued

until the viscosity of the dispersion is approximately that of the original slurry at 90°-95° C. The dispersion is then cooled rapidly to room temperature for use.

Dispersion of dialdehyde starch slurries under the conditions described normally requires from 20 to 40 minutes depending upon the lot of dialdehyde starch used. A preliminary laboratory check on how long heating is required for adequate dispersion will show the proper handling conditions for each lot of dialdehyde starch.

Wet End Addition

The wet end addition of anionic dialdehyde starch-sodium bisulfite dispersions requires the presence of additives which are substantive to cellulose in order to obtain an effective level of retention. Recently reported work (1), describing the use of papermakers' alum [$Al_2(SO_4)_3 \cdot 18\ H_2O$] for this prupose, showed that the most efficient retention of dialdehyde starch was obtained using 11 percent alum (based on dry fiber weight). Subsequent work is based on the use of combinations of dialdehyde starch and cationic starches* both in the absence and in the presence of small amounts of alum. Use of cationic starch with dialdehyde starch offers further operating advantages as well as significant increases in dry strength over those obtained using dialdehyde starch alone with alum. However, when alum is used the water must have sufficient hardness to form the proper complex. Hardness should be in the range of 300-350 p.p.m. as calcium carbonate. Sodium bicarbonate may be added to obtain this hardness. The performance of cationic starches is not significantly affected by the degree of water hardness.

The preparation and testing of handsheets has been the chief method for evaluation of the variables connected with the use of dialdehyde starch in paper. In addition a number of experimental machine trials (on a 9-inch Fourdrinier-type machine) have been made at this Laboratory. In such trials dialdehyde starch and the retention aids are ordinarily added in the chest but could presumably be added at other convenient places prior to sheet formation. In Table I are given the data obtained with both handsheets and machine runs. The machine trial data are to be considered only tentative as this work is still in progress, but are included to indicate results which might be expected in industrial operations.

* Data in this report are typical of results obtained using Cato 2 and Cato 8 manufactured by National Starch and Chemical Corporation and Keotac 22-5 produced by The Hubinger Company, all of which are commercial products. Other companies have cationic starches under development which may be equally suitable when commercially available. These products and companies are mentioned as part of the exact experimental conditions. Naming them does not constitute an endorsement of these products over those of other manufacturers.

Table I

Effects of Dialdehyde Starch in Wet End Paper Applications

Level of application Percent of dry fiber weight			Tensile strength lbs./in.		Percent wet strength	Percent dry strength increase
Dialdehyde starch	Cationic starch	Alum	Dry	Wet[a/]		
			Handsheet trials			
0	0	0	26.4	0.8	3	-
0.5	0	11	33.4	6.6	20	27
2.5	0	11	35.8	8.6	24	36
2.5	2.5	-	38.2	8.3	22	45
2.5	1.5	2	37.3	7.9	21	41
			Machine trials			
0	0	0	32.8	0.9	3	-
2.5	-	11	42.5	13.9	33	30
0.5	2.5	-	38.8	8.3	21	18
2.5	2.5	-	47.0	13.1	28	43

a/ Determined after immersion in distilled water at 23° for 1/2 hour.

The quantity of dialdehyde starch retained in the sheet may be determined by a colorimetric procedure in which the dialdehyde starch is converted to hydrazones with p-nitrophenyl-hydrazine (5).

Operating Conditions

Procedure 1. A typical operation with dialdehyde starch-bisulfite dispersion and cationic starch for the preparation of paper handsheets is as follows:

To a tap water slurry containing 340 parts of water and 1.2 parts (dry basis) of bleached softwood sulfate pulp (S.R. freeness of 700 ml.) is added an aliquot of a 3-percent aqueous dispersion of cationic starch sufficient to obtain a final slurry containing 0.030 part of cationic starch (equivalent to 2.5 percent of the dry fiber weight). After stirring for several minutes an aliquot of the dialdehyde starch-bisulfite dispersion containing 0.030 part of dialdehyde starch is added. The slurry is stirred for an additional 5 minutes and the pH of the slurry is then adjusted to 4.5 with hydrochloric acid. The sheets are formed according to TAPPI standard procedures T 205 m-53 and T 402 m-49. Additional curing by a heat treatment or storage is not necessary as maximum wet strength is developed during normal drying operation.

Procedure 2. A high degree of wet strength can also be obtained using dialdehyde starch alone with alum as the retention aid. A typical procedure is as follows:

Adjust to approximately pH 6 with hydrochloric acid, 340 parts by weight of a tap water slurry (300 p.p.m. total hardness as calcium carbonate) containing 1.2 parts (dry basis) of bleached sulfate pulp of S.R. freeness of 700 ml. To the slurry add 0.13 part of alum (11 percent of the dry weight of the pulp) and adjust the pH to approximately 4.5 with sodium bicarbonate or hydrochloric acid if this pH has not been attained through addition of the alum solution. After the slurry is agitated for several minutes introduce an aliquot of dialdehyde starch-bisulfite dispersion equivalent to 0.030 part of dialdehyde starch (2.5 percent of the dry weight of the pulp) and thoroughly mix with the pulp slurry at approximately pH 4.5 for several minutes. Form the sheets on the wire, press and condition the sheets, using diluent water at pH 4.5 and according to TAPPI standards cited previously.

Procedure 3. Combinations of dialdehyde starch and cationic starch with addition of a small amount of alum have also been found to be effective as a retention aid for the dialdehyde starch-bisulfite dispersions.

The procedure is the same as that outlined for the use of alum (Procedure 2) except that 0.018 part of cationic starch and 0.024 part of alum (1.5 and 2.0 percent, respectively, on the dry fiber weight) are substituted for the 11 percent alum. Following the addition of cationic starch, the alum is added, and the pH adjusted to 4.5 with hydrochloric acid.

Surface Application

A 2- to 10-percent dispersion of dialdehyde starch in bisulfite solution may be applied at the size press for surface sizing (2). Almost as effective is a dialdehyde starch-borax solution which is prepared by heating water containing 3 percent of dialdehyde starch and 0.05 percent borax at 70° C. for approximately 30 minutes. This solution, however, should not be applied at the wet end.

Wet Strength and Broke Recovery

Dialdehyde starch develops a degree of permanence of wet strength in paper which is more than adequate for most applications, and, in addition, allows for convenient broke recovery.

The relative permanency of wet strength of paper prepared through wet end addition of dialdehyde starch is shown in Table II and in a prior publication (2) on surface sizing.

Table II

Soaking time,* minutes	Percent wet strength
0.5	31
5	29
30	23
60	23
180	22
16 hours	16

*In distilled water at 23° C.

Wet and dry broke can easily be repulped by agitation in hot water (85° C.) for approximately 30 minutes. Soaking for 1 hour at room temperature at pH 11 is also satisfactory.

Storage Stability and Handling

Aqueous dispersions of dialdehyde starch are stable at room temperature for considerable periods of time and stability can be extended by storage in cool areas. They are not subject to normal fermentative degradation.

Dry powdered dialdehyde starch is stable indefinitely. It ages on storage and becomes more difficult to disperse in bisulfite solution. However, this problem is readily overcome by extending the time of dispersion at 90°-95° C. as indicated. Although commercial dialdehyde starch is non-volatile and practically odorless, the dust may be irritating to mucous membranes of the nose and eyes (3). Appropriate respirators should be used by operators handling the dry powder.

References Cited

1. High Wet Strength Paper by Wet End Addition of Dialdehyde Starch. B. T. Hofreiter, G. E. Hamerstrand, C. L. Mehltretter, W. E. Schulze, and A. J. Ernst. Presented at the 45th TAPPI Annual Meeting in New York, N. Y., February 22-25, 1960.

2. Periodate Oxystarches in Paper Application. Edward J. Jones, Bette Wabers, John W. Swanson, C. L. Mehltretter, and F. R. Senti. TAPPI 42(10): 862-866. 1959.

3. Technical Bulletin No. 6-129, "Sumstar™ dialdehyde starch." Miles Chemical Company, Elkhart, Indiana.

4. Technical Information, "Dialdehyde Starch." Abbott Laboratorys, North Chicago, Illinois.

5. Colorimetric Method for Determining Dialdehyde Content of Periodate Oxidized Starch. C. S. Wise and C. L. Mehltretter. Anal Chem. 30: 174-175 (1958).

Reprints may be obtained for references 2 and 5 and, after publication, for reference 1 from the Northern Utilization Research and Development Division, U.S. Department of Agriculture, Agricultural Research Service, Peoria, Illinois.

CA-N-11
May 1960

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

MICROBIAL POLYSACCHARIDES

INFORMATION ON PHOSPHOMONOESTERS OF MANNOSE POLYMERS

Phosphomonoesters of mannose polymers have resulted from further research on the chemical and physical properties of members of a family of microbial polysaccharides recently developed at this Division.*

Nature and Origin: These products are derived from phosphomannans by mild acid hydrolysis of the cross-linking pyrophosphate bonds. Phosphomannans are high molecular weight potassium salts of pyrophosphorylated mannans produced extracellularly by certain primitive yeasts of the genus *Hansenula* and related genera.

Preparation:

A. Phosphomannans: They are produced fermentatively as described in a previously issued information sheet.

B. Phosphomonoesters of Mannose Polymers: Phosphomannan solutions are made acidic either by decationization with sulfonic acid-type cation exchange resins or by adjustment to pH 2.5 with mineral acid. The acidic solutions are then heated 30 minutes at 90°-100° C. Solutions thus prepared may be neutralized and diluted to desired concentration prior to use.

Composition and Structure: All the phosphorous in the high molecular weight phosphomannan gums occurs in the form of acid-stable mannose-6-phosphate residues. The phosphate ester residues are all cross-linked through acid-labile pyrophosphate linkages. Whereas only primary phosphoryl groups are present in the intact phosphomannans, mild acid hydrolysis liberates an equivalent amount of secondary phosphoryls as illustrated in Figure 1. Conditions of mild acid hydrolysis completely liberate secondary phosphoryl groups but cause little concommitant splitting of mannosidic linkages (Table 1). Thus, the products of mild hydrolysis are phosphoric acid monoesters of mannosidic polymers.

Types of Product Potentially Available: Characteristic phosphomannans are produced by different species of yeast. These vary in degree of phosphorylation and in average length of mannosidic chains. In Table 2, phosphorylation is expressed in terms of the mannose to phosphorous molar ratio (M:P). Apparent chain length is designated as degree of polymerization (D.P.) of mannose units. Depending upon the parent phosphomannans selected, a variety of phosphomonoesters can be produced.

Properties: Clear, nonviscous, odorless, colorless, and tasteless solutions.

* Information on Phosphomannan Y-2448, CA-N-7, October 1958.
Information on Polysaccharide B-1459, CA-N-9, September 1959.

Note: Recent work has shown that the secondary phosphoryl cross-linkages are of the hemiacetal phosphate type and not pyrophosphate.

Dispersant Action: Table 3 illustrates the dispersant effects of phosphomonoesters on 50% zinc oxide suspensions.

Table 1. Mild Acid Hydrolysis of Decationized Phosphomannan Solutions
(Values in microequivalents per ml. of 1% solution.)
(Conditions: 20 minutes at 100° C.)

Phosphomannan	Total P	Phosphoryl		Increase in Reducing Equivalents
		Primary	Secondary	
Y-2448	8.2	7.4	6.2	0.4
Y-1842	16.8	13.7	14.2	1.2

Table 2. Properties of Certain Yeast Phosphomannans

Organism	NRRL No.	M:P[1]	D.P.[2]	Viscosity of 0.5% Solution[3]
				Centistokes
Hansenula capsulata	Y-1842	2.5	244	33
Hansenula holstii	Y-2448	5.7	588	46
Torulopsis pinus	Y-2023	8.4	51	71
Saccharomyces pini	Y-2579	13.0	50	83
Hansenula minuta	Y-411	27.5	144	11

[1] Mannose:Phosphorous molar ratio.

[2] Apparent degree of polymerization of mannosidic chains.

[3] Kinematic viscosities measured at 20° C. Derived phosphomonoesters have essentially no viscosity.

Table 3. Dispersant Effect of Phosphomonoesters on Zinc Oxide Suspensions

Mixture[1]	Viscosity in Centipoises[2]			
	6 r.p.m.	12 r.p.m.	30 r.p.m.	60 r.p.m.
A	<100,000	(Too viscous to read even at lowest spindle r.p.m.)		
B	<100,000	(Too viscous to read even at lowest spindle r.p.m.)		
C	2,520	1,800	1,400	872
D	2,800	1,840	940	560

[1] A. 30 g. ZnO powder + 30 ml. H_2O
B. 30 g. ZnO powder + 30 ml. 1% NaCl
C. 30 g. ZnO powder + 30 ml. 1% Y-1842 phosphomonoester
D. 30 g. ZnO powder + 30 ml. 1% Y-2448 phosphomonoester

[2] Brookfield viscometer readings (No. 3 spindle). This equipment is named merely as part of the exact experimental conditions. Mentioning it does not constitute an endorsement of this apparatus over those of other manufacturers.

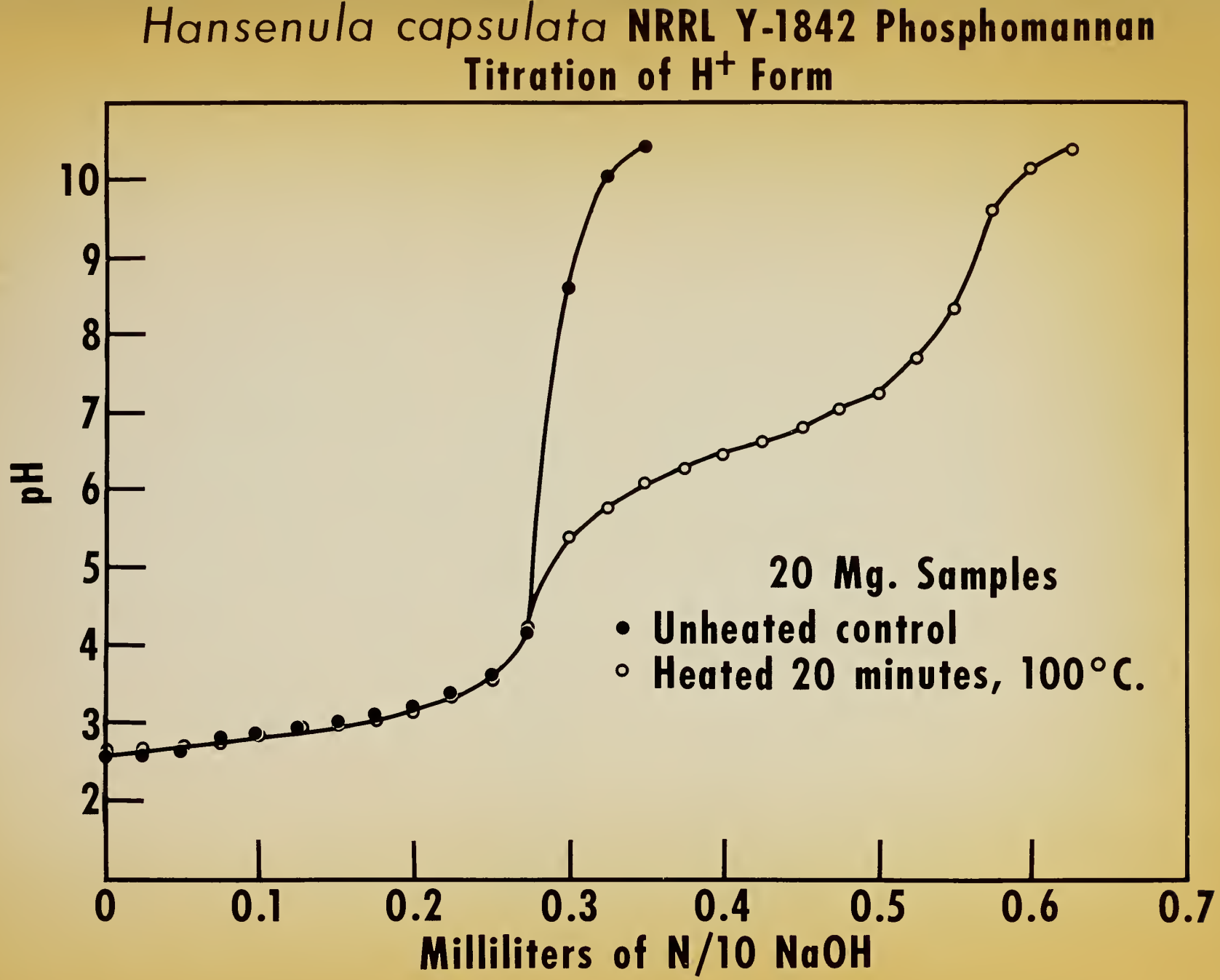

Hansenula capsulata NRRL Y-1842 Phosphomannan
Titration of H^+ Form
pH
10
9
8
7
6
5
4
3
2
20 Mg. Samples
• Unheated control
○ Heated 20 minutes, 100°C.
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Milliliters of N/10 NaOH

Fig. 1

CA-N-12
October 1960

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

MICROBIOLOGICAL PRODUCTION OF CAROTENOIDS

INFORMATION ON MICROBIAL *BETA*-CAROTENE

All-*trans*-β-carotene can be readily produced by a unique fermentative process involving mating of opposite types of the heterothallic mold, *Blakeslea trispora* (1-4). Average yields of 40,000 µg of carotene/100 ml. of medium (5 percent solids) or 8,000 µg of carotene (0.8 percent)/gram of dry solids were reported earlier (5,6,7). Continuing research has led to a reproducible increase in these yields to approximately 86,000 µg of carotene/100 ml. of medium or approximately 17,000 µg of carotene (1.7 percent)/gram of dry solids. These increased yields result from the addition of a hydrocarbon solvent to the fermentation medium. Research is still in progress; problems on tank-scale production have not yet been investigated.

CULTURES

Initial investigation used mating strains of *B. trispora* NRRL 2456 (+) and NRRL 2457 (-). Some difficulty was experienced with degeneration of these cultures. In subsequent experiments two more efficient mating strains of *B. trispora* NRRL 9216 (+) and NRRL 9159 (-) have been employed. These strains are maintained on potato dextrose agar (PDA). Six-day-old cultures are used to inoculate culture media.

INOCULUM

The following medium is inoculated from 6-day-old PDA slants:

Medium: (150 ml./500 ml. Erlenmeyer flask)

Acid-hydrolyzed soybean meal	4.7 percent
Acid-hydrolyzed corn	2.3 percent
Thiamin hydrochloride	2.0 mg./liter
Water	
pH adjusted to 6.2-6.5 with sodium hydroxide before sterilization	

Plus and minus strains are grown separately for 2 days at 28° C. on a rotary shaker operating at 200 r.p.m. Five percent of each strain is added to the fermentation flask.

FERMENTATION

Medium: (100 ml./500 ml. Erlenmeyer flask)

The fermentation medium as described contains, in addition, 5 percent animal fat, 0.12 percent nonionic detergent, and 5 percent solvent*; 0.1 percent β-ionone is added aseptically after 48 hours of incubation at 28° C. on a rotary shaker operating at 200 r.p.m. Procedures for harvesting and analysis have been previously described (2,3).

Results: Repeated experiments indicated that a concentration in the medium of 5 percent animal fat and 5 percent solvent gave the best results (Table 1). Increasing either the concentration of lipide or solvent, or both, did not result in substantially increased carotene yields. Maximum yields were attained after approximately 6 days of fermentation in both controls and flasks containing 5 percent solvent (Figure 1). Addition of solvent after 1 to 3 days of fermentation also resulted in increased yields which, however, were usually lower than those achieved by solvent addition at initiation of fermentation.

Table 1

Influence of Animal Fat and Solvent on Carotene Yield

Animal fat	Solvent[1]	Mycelium dry weight/100 ml.	Carotene yield in solids		Carotene yield /100 ml.
Percent	Percent	Gram	Percent	µg/gram	µg
3	0	3.95	0.58	5,750	22,700
4	0	4.77	0.68	6,805	33,700
5	0	5.97	0.90	9,000	53,850
6	0	6.54	0.67	6,667	43,700
7	0	7.25	0.57	5,717	41,470
8	0	8.31	0.48	4,750	39,800
3	5	3.27	1.3	13,133	42,830
4	5	3.93	1.5	15,300	61,570
5	5	4.95	1.7	17,000	84,000
6	5	5.64	1.6	15,500	87,230
7	5	6.42	1.1	10,750	69,200
8	5	5.97	0.93	9,300	56,570

[1]Deobase

*Data in this report are typical of results obtained using Deobase manufactured by L. Sonneborn Sons, New York. Other companies have products which may be equally suitable. This product or others mentioned are cited as part of the exact experimental conditions. Naming them does not constitute an endorsement of these products over those of other manufacturers.

STABILIZATION OF β-CAROTENE

Storage tests were conducted on the stabilization of carotene produced intracellularly by the mated cultures of *B. trispora*. Addition of 0.25 percent Santoquin (6-ethoxy-2,2,4-tri-methyl-1,2-dihydroquinoline) to the medium during fermentation, based on a predicted yield of 5 percent solids or of the dried product, was more effective in stabilizing β-carotene; a half-life of about 10 weeks resulted (Figure 2). Suspension of dried fermentation solids in vegetable oil also proved effective in protecting carotene from oxidation; a half-life of about 12 weeks was obtained (Figure 2).

REFERENCES CITED

1. Hesseltine, C. W., and Anderson, R. F. 1957. Microbial Production of Carotenoids. I. Zygospores and Carotene Produced by Intraspecific and Interspecific Crosses of Choanephoraceae in Liquid Media. Mycologia 49, 449-452.

2. Anderson, R. F., Arnold, Margie, Nelson, G. E. N., and Ciegler, A. 1957. Microbial Production of Carotenoids. II. The Effects of Selected Medium Adjuncts on *Beta*-Carotene Synthesis. Abstracts, p. 17A, 132nd Meeting, American Chemical Society, Division of Agricultural and Food Chemistry, New York, N. Y.

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Fig. 1

Effect of Solvent (Deobase) on Rate of Carotene Production

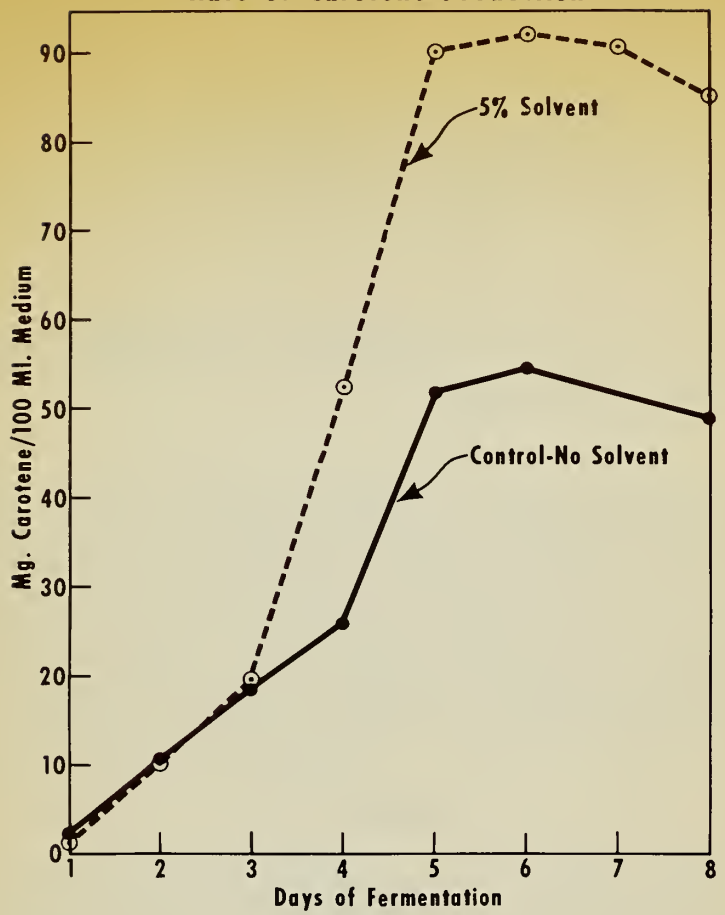
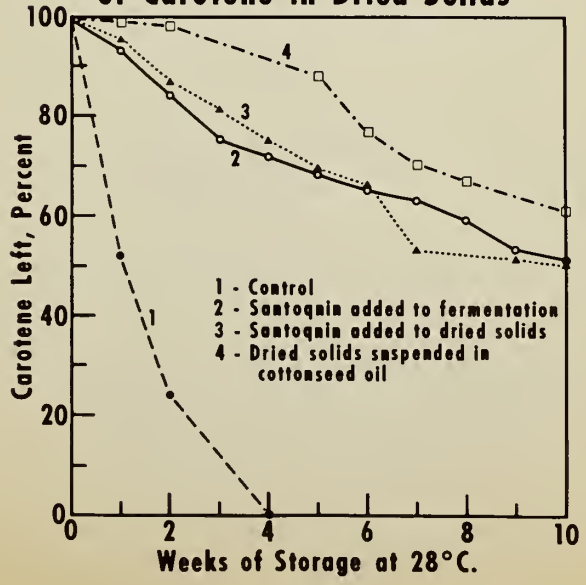


Fig. 2

Effect of Antioxidant on Stability of Carotene in Dried Solids



CA-N-12
October 1960

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

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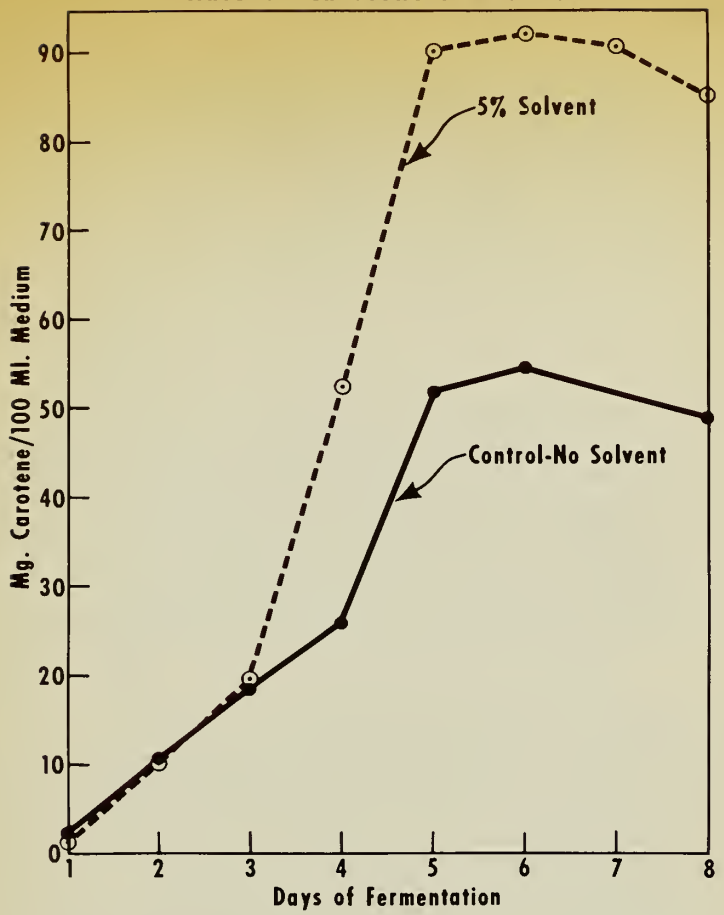

Fig. 2

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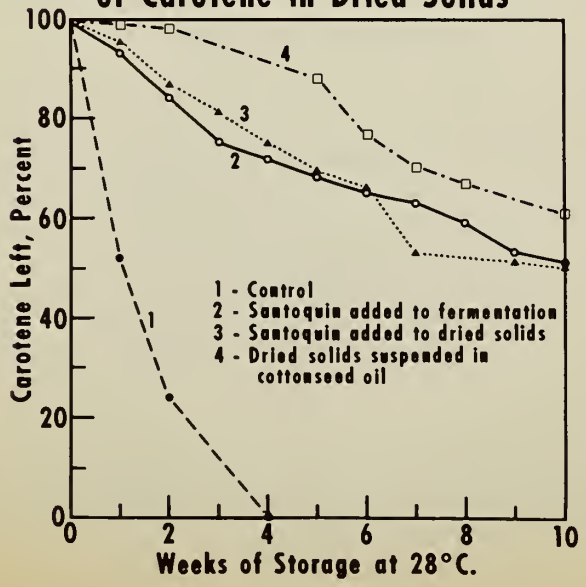

CA-N-13
January 1961

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

SOYBEAN MILK

Soybean milk is reported to have originated in China before the Christian Era. Although soybeans are a popular source of food for Oriental people, the milk is one of the minor uses, perhaps because of the characteristic flavor. At present there is no significant production of soybean milk in Japan, but recently the Japanese have revived interest in its possibilities for feeding babies and research has been initiated on improving its quality. The largest soy milk plant has been built in Indonesia by UNICEF (United Nations International Children's Fund). A privately owned plant is known to be operating successfully in Hong Kong, and there is much interest in and research for a vegetable milk in India.

Soybean milk was first produced in the United States at Mt. Vernon, Ohio, about 1940 by Dr. Harry W. Miller, a former medical missionary to China. The Mt. Vernon plant is now owned by the Loma Linda Food Company,* which also has another vegetable food plant and headquarters at Arlington, California. Other companies known to be marketing soybean milk are The Borden Company, Mead-Johnson and Company, and Worthington Foods, Inc. Soybean milk is sold mainly through drug and specialty food stores. Its largest market in the United States is for babies who are allergic to cow's milk. We have an unverified report that about 7½ percent of all babies in the U.S. have this allergy.

There is no published information on the production capacity for soy milk in the U. S., but it compares favorably with other important food uses of soybeans.

METHOD OF MAKING SOYBEAN MILK

There is no standard method for making soybean or vegetable milk and its composition varies widely. The original method used in China is simple; the beans are washed, soaked overnight in water, and ground in a stone mill; the resulting mash is extracted with water at a ratio of about 8 parts of water to 1 part of dry soybeans. Protein and oil are extracted in about the same ratio as they occur in the beans and are in the form of a very stable emulsion. Undissolved solids are removed with a coarse cloth filter, and the milk is boiled for at least 20 minutes. Heating improves both the flavor and nutritional value of the milk. Table 1 shows the composition of milk prepared by this general procedure.

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*Mention of company names does not constitute an endorsement of their products over those of others in the field, nor guarantee that the list is necessarily complete.

Table 1

Composition of Soybean Milk Compared with Cow's Milk

Kind of milk	Water	Protein	Fat	Carbohydrate	Other substances	Ash	Total solids	Solids not fat
	%	%	%	%	%	%	%	%
Soybean	92.00	3.70	2.00	1.80		0.50	8.00	6.00
Soybean	90.00	4.95	2.97	1.34		0.44	9.70	6.73
Soybean	89.25	3.15	3.10	3.02	1.02	0.45	10.74	7.64
Soybean	92.50	3.02	2.13	0.03	1.88	0.41	7.47	5.34
Cow	87.30	3.42	3.67	4.78		0.73	12.60	8.93

Source: "The Soybean" by C. V. Piper and W. M. Morse, Peter Smith, New York City (1943), p. 230.

The modern trend in manufacturing vegetable milk is to fortify it with a vegetable oil for adjusting the ratio of protein and fat to either that of cow's milk or of mother's milk and to fortify it further with vitamins and with such minerals as calcium, iron, and potassium. The characteristic beany flavor of most soybean milk preparations may be partially masked with sugar or with such flavors as vanilla, chocolate, and malt.

The final product is homogenized and sold as either a fluid milk or is concentrated by vacuum evaporation and canned to make a condensed milk, or is spray-dried to form a powder. Although most soybean milks are water extracts of the whole soybean as described, there has been a special grade of soy flour packaged and sold in the U.S. as soybean milk. This product may contain added minerals and vitamins but is only partly soluble in water.

Very recently an alternate method of making soybean milk has been developed. This new method starts with water-soluble soybean protein and makes an emulsion by adding emulsifiers, oil, minerals, vitamins, and sugars. Although more expensive than extracting the whole bean, this method permits a much better control of the composition and concentration of the milk; also, the milk has less beany flavor than the whole bean extract.

In extracting whole beans for making milk, the yield of solids will be in the range of 50 to 55 percent based on dry weight of the beans. Extracted solids from 1 bushel of beans make more than 100 quarts of soybean milk. This estimate does not include the materials added in making a fortified milk.

NUTRITIONAL VALUE AND USES

Since there is no standard composition for soy milk or standard method for its production, its nutritional value varies. Some have reported that it causes diarrhea or other intestinal disturbances in very young infants. Other experimenters have reported that they have not encountered this problem with children over three months old.

Whole protein of the soybean has about the same nutritional value as casein, and soybean oil appears adequate for humans. The first limiting amino acid for soybean protein is methionine, but lysine is present in more than sufficient amounts. Sesame protein has a deficiency of lysine and an excess of methionine; thus, these two proteins complement each other in these two essential amino acids and have been used together to a limited extent for making a vegetable milk. Peanuts also have been used with soybeans in making a vegetable milk.

Unknown factors in using natural soybean milk appear to be constituents other than protein and oil, such as saponins, isoflavone glucosides, and other biologically active components of the bean; however, the exact factors will not be established until the milk has been more extensively investigated. Research on compositional, nutritional, and biological effects of soy milk on children and animals remains to be carried out to clarify contradictory reports and to determine how soy milk should be prepared and used.

Feeding results on children and animals have been from moderately good to excellent. These results suggest that standardized formulas and methods of production can be developed that will meet the requirements for weaning babies and feeding children in overpopulated countries.

SELECTED REFERENCES

1. Barnes, G. R., Jr. 1959. Acceptance of a Soya Food by Infants. Amer. Jour. Dis. Children *98*, 1-5.

2. Chang, Irene C. L., and Murray, Hazel C. 1949. Biological Value of the Protein and the Mineral, Vitamin, and Amino Acid Content of Soymilk and Curd. Cereal Chem. *26*, 297-305.

3. De, S. S., and Subrahmanyan, V. 1946. Processing of Soybean for the Production of Milk. Current Sci. [India] *14*, 204-205; Chem. Abs. *40*, 407.

4. Fomon, S. J. 1959. Comparative Study of Human Milk and a Soya Bean Formula in Promoting Growth and Nitrogen Retention by Infants. Pediatrics *23*, 577-584.

5. _____ and May, C. D. 1959. The Adequacy of Soya Bean Protein in Promoting Nitrogen Retention in Infancy. Amer. Jour. Dis. Children *98*, 6-10.

6. Karnand, B. T., De, S. S., and Subrahmanyan, V. 1948. Fortification of Soybean Milk with Calcium and Study of Its Availability to Young Growing Rats. Indian Jour. Med. Res. *36*, 349-354; Chem. Abs. *48*, 4869 (1954).

7. Miller, H. W. 1959. Why Japan Needs Soy Milk. Soybean Dig. *19*(6), 19.

8. _____1960. The Package Soy Milk Shop. Soybean Dig. *20*(12), 14.

9. Nandi, D. K., Rajagopalan, R., and De, S. S. 1953. Vegetable Milk. I. Process and Nutritive Value. Indian Jour. Physiol. and Allied Sci. *7*, 1-5; Chem. Abs. *48*, 904 (1954).

10. Ney, L. F. 1958. Growth and Reproduction of Rats on Diets of Evaporated Milks and a Vegetable Fat Milk Product. Jour. Agr. Food Chem. *6*, 223-227.

11. Smith, A. K., and Beckel, A. C. 1946. Soybean or Vegetable Milk. Chem. Engin. News *24*, 54-56; AIC-113, processed, 14 pages.

12. Subrahmanyan, V., Reddy, S. K., Moorjani, M. N., and others. 1954. Supplementary Value of Vegetable-Milk Curds in the Diet of Children. Brit. Jour. Nutr. *8*, 348-352.

Reference 11, part 2, may be obtained from the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois. AIC-113 contains a bibliography of literature on soybean milk from 1896 through 1944.

CA-N-14
April 1961

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

MICROBIAL POLYSACCHARIDES

INFORMATION ON POLYSACCHARIDE Y-1401

This product, from the research efforts of a team of microbiologists, carbohydrate chemists, and chemical engineers, is one of a series of polysaccharides made by fermentation processes developed at this Division.*

Information presented here is preliminary and is subject to further confirmation and extension as current research progresses and as industrial evaluation justifies. At present, research on this polysaccharide has been restricted to preparing samples of a representative product and obtaining a few fundamental characterizations.

Nature and Origin: A high molecular weight, heteropolysaccharide produced extracellularly by the yeast, *Cryptococcus laurentii* var. *flavescens* NRRL Y-1401.

Preparation: Pure culture fermentation of a medium containing 4-6 percent commercial glucose, an organic nitrogen source such as autolyzed brewer's yeast, and inorganic salts. Incubation time 5 days at 25° C., aerobic conditions.

Purification and Isolation: Centrifugation to remove cells, precipitation by methanol in the presence of electrolyte such as potassium chloride, reprecipitations, and finally dehydration of the cohesive gum by methanol. Other methods of purification are under investigation. Yields of purified product under experimental conditions to date, have been about 20-30 percent based on *D*-glucose.

Composition: Polysaccharide Y-1401 contains *D*-mannose, *D*-xylose, *D*-glucuronic acid (as the potassium salt) and acyl in the approximate molar ratio of 4:1:1.5. The acyl groups, presumably acetyl, are in *O*-ester form. The content of glucuronic acid is about 23 percent and of acyl about 7. Potassium chloride and nitrogen, as extraneous matter, amount to about 0.15 percent each.

Properties:

Gum.--Rather dense, cohesive, short flow characteristics, but rubbery when compacted.

Solid.--A white powder--absorbs about 14 percent moisture when equilibrated under conditions of 20° C. and 50 percent relative humidity. A humidified sample stored 2 years under these conditions showed no change in viscosity or in acyl content.

* Information on Phosphomannan Y-2448, CA-N-7 October 1958.
Information on Polysaccharide B-1459, CA-N-9, September 1959.
Information on Phosphomonoesters of Mannose Polymers, CA-N-11, May 1960.

In water, solid particles solvate to a dense gum which disperses somewhat slowly, but completely.

Aqueous Solutions.--Somewhat opalescent, thixotropic, and show soft gelation. Concentrated solutions (such as 1.5 percent) do not adhere to glass. The pH is in the range 6.0 to 7.0 for concentrations 0.1 to 1.5 percent.

Specific Rotation.--+38° (*c*, 0.5 in water or 0.1*M* KCl).

Viscosity Relationships*: The viscosity-concentration curve for Polysaccharide Y-1401 in water is shown in Figure 1. For comparison, curves are included on commercial grade guar gum and high-viscosity sodium alginate and on our Polysaccharide B-1459.

Percentage changes in viscosity resulting when potassium chloride is added to water solutions differing in polysaccharide concentration, are shown in Figure 2. Essentially the same behavior occurs when either borax or calcium chloride is substituted for potassium chloride.

For comparison with the result shown in Figure 2 for 1 percent Polysaccharide Y-1401 in presence of 1 percent potassium chloride, percentage changes obtained under identical conditions for other hydrocolloids are as follows: Our bacterial Polysaccharide B-1459, +55; high-viscosity sodium alginate, -30; gum tragacanth, -50; gum karaya, -90.

The plot of viscosity *vs.* rate of shear (Figure 3) indicates that the solutions have plastic rheological characteristics.

Viscosity of 1 percent solutions is essentially constant between pH 5 and 7 and changes little between pH 4 and 11.5. Solubility does not decrease at acid or basic pH's. Unbuffered solutions adjusted approximately to initial pH's above 4.2 and below 12.2 decrease slowly in pH with time (Table 1). Corresponding viscosity values decrease somewhat when the initial pH is acidic, but increase when it is basic through pH 11.5.

Unbuffered aqueous solutions of Polysaccharide Y-1401 decrease moderately in viscosity (measured at 25° C.) after heating. Decrease in viscosity with heat is only slightly greater in the presence of potassium chloride. Decreases are partially reversible with time at 25° C.

Deacylated Polysaccharide Y-1401: Saponification of acyl groups probably causes the neutralization of alkali indicated in Table 1. Acyl groups are removed quantitatively by 0.01*M* alkali under oxygen-free conditions. The product shows essentially the same viscosity characteristics in relation to concentration and salt as the native polysaccharide, but it requires a lower percentage concentration of alcohol for precipitation from aqueous solution.

Applicability: Polysaccharide Y-1401 has several properties which might provide a basis for industrial applications. These are its homogeneous, highly viscous solutions, the relatively moderate influence of mono- and divalent cations upon its solution viscosity, and the difference in compatibility of aqueous solutions of its native and deacylated forms with organic liquids such as alcohols.

* All viscosity data were obtained at 25° C. with a Brookfield viscometer operating at 30 r.p.m. This equipment is named merely as part of the exact experimental conditions. Naming it does not constitute an endorsement of this apparatus over those of other manufacturers.

Fig. 1

Viscosity-Concentration Relationships

Polysaccharide Y-1401 Compared with Other Polysacolloids.

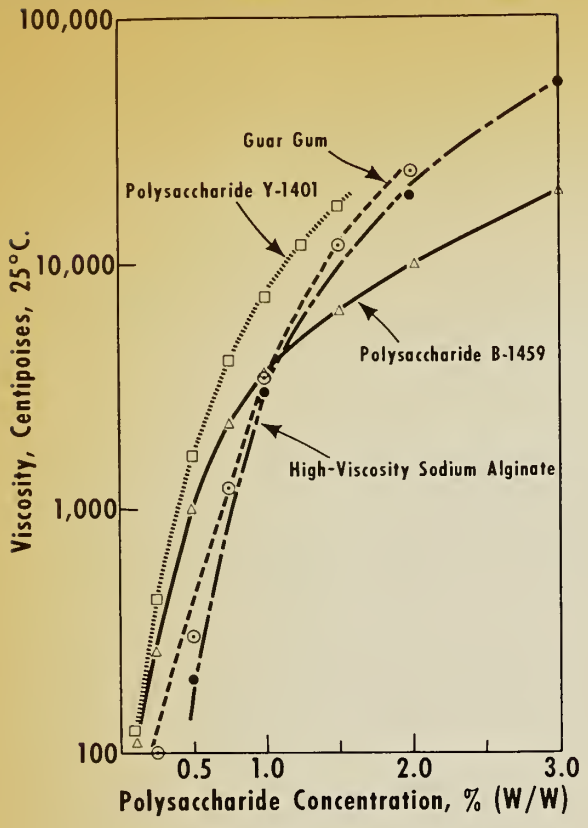


Fig. 2

Polysaccharide Y-1401

Percentage Change in Viscosity of Aqueous Solutions upon Addition of Potassium Chloride.

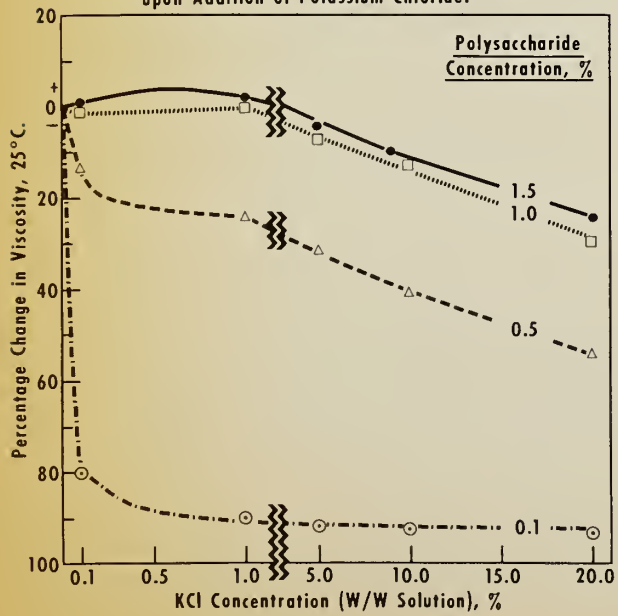


Fig. 3

Polysaccharide Y-1401

Viscosity of 1 Percent Solution vs Rate of Shear.

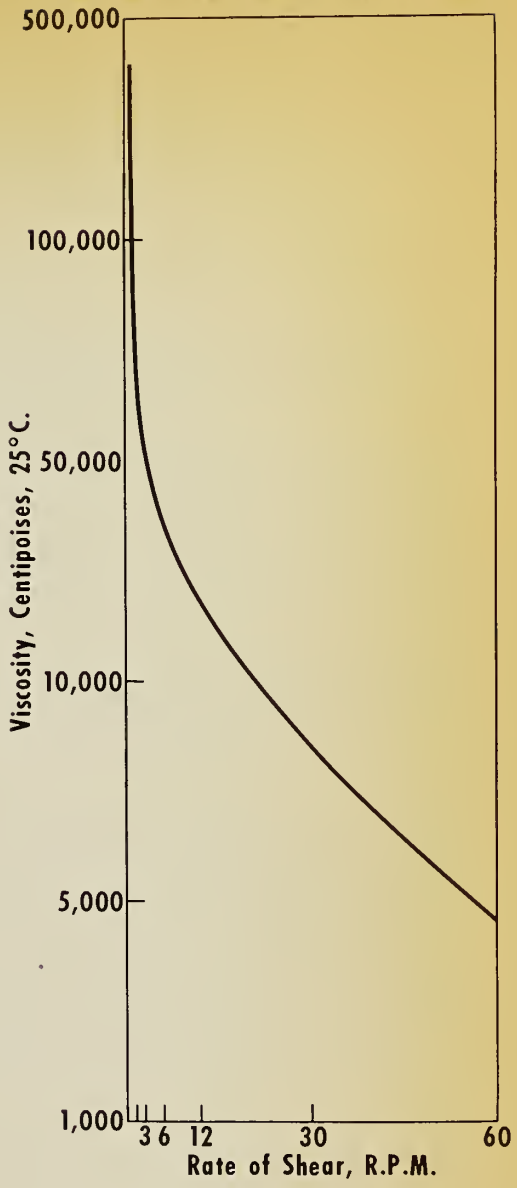


Table 1.

Relation of Initial pH of Unbuffered Solutions to Changes in pH and Viscosity with Time at 25°C.

Sample Number	Time, hours					
	0	24	72	0	24	72
	pH			Viscosity, cps.		
A	2.9	2.9	2.9	840	840	700
B	4.2	4.2	4.2	1,520	1,480	1,400
C	7.0	6.5	6.3	1,780	1,680	1,680
D	10.9	7.7	6.7	1,500	1,720	1,740
E	11.5	7.9	6.7	1,400	1,740	1,740
F	12.2	11.9	11.9	1,200	900	740

CA-N-15
May 1961

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

FATTY ACIDS

INFORMATION ON LINOLENIC ACID

Linolenic acid of high purity is a new product with industrial potential resulting from engineering studies on the separation of vegetable oil fatty acids by liquid-liquid extraction in a commercial-type centrifugal extractor. This product is one of a series of high purity fatty acids made from vegetable oils produced from farm crops grown in the United States.

Nature and Origin: Linolenic acid--*cis, cis, cis*-9,10-12,13-15,16-octadecatrienoic acid, molecular weight 278--occurs naturally in soybean, linseed, and perilla oils where it is combined with glycerol. It comprises 50 to 60 percent of the fatty acids of linseed oil.

Preparation and Purification: Linseed oil is hydrolyzed by treatment with water at a high temperature and pressure (approx. 260° C., 750 p.s.i.) to yield glycerol and free fatty acids. Commercially, hydrolysis is generally conducted by a continuous countercurrent process. The fatty acids are fractionated by liquid-liquid extraction in a Podbielniak* centrifugal extractor to yield a 50-percent fraction containing linolenic acid. Extraction is conducted with furfural, containing 2.5 percent water, and hexane, at a temperature of 110° F. The furfural phase from the extractor, containing the linolenic acid, is evaporated under vacuum (2-20 mm.) to remove the solvent, and the resulting fatty acids are distilled at high vacuum (1-2 mm.) to purify them.

Properties: The clear, yellow linolenic acid contains 95 to 97 percent linolenic acid with a small amount of linoleic acid.

Color (Gardner Scale)--about 5 to 6.

Iodine Value--265 to 268.

Stability--oxidizes slowly at room temperature.

Linolenic Acid--should be stored under nitrogen or at 0° C. if oxidation is to be avoided.

* Mention of company names does not necessarily imply endorsement of their products by the U. S. Department of Agriculture.

SUGGESTED USES

1. Coatings: Excellent alkali resistance, fast dry, good gloss, and film hardness--useful in high-gloss finishes. Have poor yellowing characteristics.

2. Trimer acids: Trimeric acid by thermal polymerization of linolenic acid potentially useful as wetting agent for asphalt and for making polyurethanes.

3. Rubber stabilizer: Esters as ozone acceptors for stabilizing rubber and plastics.

4. Ore flotation: Collector for oxide ores.

5. Corrosion: Hexadecylamine salt as a corrosion inhibitor.

6. Low pour-point saturated acids: Thermal treatment of linolenic acid in the presence of excess alkali and a suitable solvent.

7. Soaps: Liquid and gel soaps for greases and metallic drier soaps.

CA-N-16
May 1961

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

FATTY ACIDS

INFORMATION ON LINOLEIC ACID

Linoleic acid of high purity is a new product with industrial potential resulting from engineering studies on the separation of vegetable oil fatty acids by liquid-liquid extraction in a commercial-type centrifugal extractor. This product is one of a series of high purity fatty acids made from vegetable oils produced from farm crops grown in the United States.

Nature and Origin: Linoleic acid, together with other fatty acids, occurs in many drying and semidrying oils combined with glycerol as triglycerides. It is *cis,cis*-9,10-12,13-octadecadienoic acid, molecular weight 280, and is found in the highest concentration in domestic safflower oil where it comprises about 75 percent of the fatty acids.

Preparation and Purification: Safflower oil is hydrolyzed by treatment with water at a high temperature and pressure (approx. 260° C., 750 p.s.i.) to yield glycerol and free fatty acids. Commercially, hydrolysis is generally conducted by a continuous countercurrent process. The fatty acids are fractionated by liquid-liquid extraction in a Podbielniak* centrifugal extractor to yield a 75-percent fraction containing linoleic acid.

Extraction is conducted with furfural, containing 2.5 percent water, and hexane, at a temperature of 100° F. The furfural phase from the extractor, containing the linoleic acid, is evaporated under vacuum (2-20 mm.) to remove the solvent, and the resulting fatty acids are distilled at high vacuum (2 mm.) to purify them.

Properties: The clear, yellow linoleic acid contains 95 to 97 percent linoleic acid with small amounts of linolenic, oleic, and palmitic acids.

Color (Gardner Scale)--about 5 to 6.

Iodine Value--174 to 177.

Stability--oxidizes slowly at room temperature. Linoleic acid should be stored under nitrogen or at 0° C. if oxidation is to be avoided.

* Mention of company names does not necessarily imply endorsement of their products by the U. S. Department of Agriculture.

SUGGESTED USES

1. Coatings: Good alkali resistance, rapid dry, and low yellowing.

2. Epoxidized esters: Stabilizer for vinyl chloride plastics and chlorinated solvents (acid acceptor).

3. Dimer acid: Thermal polymerization of linoleic acid to give a high yield of dimeric acid for use in polyamide resins.

4. Diels-Alder adducts: Reaction with dienophiles to give polyfunctional products.

5. Rubber stabilizer: Esters as ozone acceptors for rubber products and plastics.

6. Biological applications: Specific ones indicated in the literature.

CA-N-18
October 1961

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

SOYBEAN PROTEIN PRODUCTS

Information on Foams and Thermoreversible Gels

A process has been developed for making a purified protein that has possibilities as a gel or foam for food products. The foams are easily prepared and are very stable. The gels are thermally reversible; upon heating they liquefy and upon cooling they revert to the gel.

The end product is prepared by an alcoholic extraction of crude soybean protein; it is a light-colored and bland-tasting product. It can be combined with sugar, flavor, and coloring materials to produce a variety of products.

Preparation and Purification

Soybean Sodium Proteinate: Undenatured flakes prepared in the laboratory by hexane extraction are extracted twice with water, first at a solvent-to-meal ratio of 10:1 and a second time at 5:1. The insoluble residue is removed by centrifugation and the supernatants of the two extracts are combined and adjusted to pH 4.5 with hydrochloric acid; the precipitated curd is recovered in a centrifuge and freeze-dried. The crude protein is then finely ground in a hammer mill. The finely powdered material is extracted with 25 volumes of 86-percent aqueous ethanol (volume:volume) in a Waring Blendor for 15 minutes. This removes a phospholipid-like material that interferes with formation of foams and gels. The extracted protein is collected by filtration and dried in a vacuum oven at 50° C. The dried, washed protein is then dispersed in aqueous sodium hydroxide at pH 7.5, centrifuged to clarity, and again freeze-dried to yield the purified soybean sodium proteinate.

Foams: The purified soybean sodium proteinate is dispersed in water (3-10 percent depending on application). The resulting suspension is heated in a boiling-water bath to 85° C., then decanted into a mixer, and whipped vigorously from 3-15 minutes. Extremely stable foams are produced. Foams can be prepared at most pH values, except in the approximate region of 3 to 6. Sugar, flavorings, salts, and coloring materials may be added with little adverse effect. By varying conditions, whips can be prepared which resemble egg whites, meringues, cake icings, divinity-type candies, and marshmallows.

Thermoreversible Gels: Aqueous dispersions containing 5-15 percent of the purified soybean sodium proteinate are prepared. The material is dissolved at room temperature or with mild heating, and any insoluble material is removed by centrifugation. The slightly amber-yellow solution

is decanted from the insolubles and heated in a boiling-water bath to around 85° C. Upon cooling the protein solution, a gel forms which becomes fluid again upon reheating. The reversibility of the gel depends, in part, on the concentration of soybean proteinate. Laboratory procedure is to disperse 10 grams of purified soybean sodium proteinate in 70 ml. of water. The solution is clarified by centrifugation, heated, and cooled to form a gel. Coloring materials, sugar, and flavorings may be added to the gels to simulate dessert or salad-type products. The use of these gels with fruits may be limited because of their acidity and the protein's insolubility at its isoelectric point, pH 4.4-4.6.

Composition and Nutritional Qualities

The amino-acid composition and protein-efficiency ratios by analysis and feeding tests have shown that the acid-precipitated protein of soybeans is of good quality. The first limiting amino acid of soybean protein is methionine, but lysine is present in more than sufficient amounts. Supplementing with a source of either methionine or cystine or both will give a high quality proteinaceous product.

Precautions and Techniques

Care should be exercised when preparing either the foaming or gelling materials. Various soybean processors use different methods of producing soybean meal and isolating soybean protein. For example, some use enough heat to denature the protein; others use bisulfite to extract the protein. This chemical may alter the protein. All these treatments affect the protein and may make the phospholipid-like material more difficult to remove by the alcoholic treatment. Attempts to extract some samples of commercially isolated soybean protein failed to yield a satisfactory foaming material. Also, we have observed that drying the water-moist protein at pH 4.5 in a forced-draft oven changes solubilities and subsequent alcohol-extraction does not perform properly.

SELECTED REFERENCES

1. Eldridge, A. C., Wolf, W. J., Nash, A. M., and Smith, A. K. Alcohol Extraction of Soybean Protein. Abstr. Papers, 23A, 140th Meeting, Amer. Chem. Soc., Chicago, Ill., Sept. 3-8, 1961.

2. Eldridge, A. C., Hall, P. K., and Wolf, W. J. Stable Foams from Unhydrolyzed Soybean Protein. Food Technol. In preparation.

3. Rackis, J. J., Anderson, R. L., Sesame, H. A., Smith, A. K., and VanEtten, C. H. Amino Acids in Soybean Hulls and Oil Meal Fractions. J. Agr. Food Chem. 9; 409 (1961).

4. Smith, A. K., and Wolf, W. J. Food Uses and Properties of Soybean Protein. I. Food Uses. Food Technol. 15; 4 (1961).

5. VanEtten, C. H., Hubbard, J. E., Mallan, Jean M., Smith, A. K., and Blessin, E. W. Amino Acid Composition of Soybean Protein Fractions. J. Agr. Food Chem. 7; 129 (1959).

6. Wolf, W. J., and Smith, A. K. Food Uses and Properties of Soybean Protein. II. Physical and Chemical Properties of Soybean Protein. Food Technol. 15; 12 (1961).

Reprints may be obtained for references 3, 4, 5, and 6 from the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

CA-N-19
January 1962

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

VEGETABLE OIL ADDUCTS

USE AS PLASTICIZERS FOR NITRILE RUBBERS

Diels-Alder adducts prepared directly from such polyunsaturated vegetable oils as soybean, linseed, and safflower, and maleic esters are excellent partially extractable plasticizers for nitrile rubbers, resembling polymeric-type plasticizers in low-temperature properties, volatility, and oil-extraction.

Nature and Origin: Reaction of oils with maleic anhydride to give succinate-type adducts has been known for many years. However, when the polyunsaturated portions of vegetable oils are conjugated and isomerized simultaneously with addition of a maleic ester, Diels-Alder adducts are formed, similar to those which can be obtained by the reaction of dienophiles with *trans,trans*-9,11-octadecadienoic acid. The actual proportion of adduct in the oil will be from 80 to 90 percent of theoretical, based on the polyunsaturated material available.

Preparation: Soybean, linseed, or safflower oil; dimethyl maleate (100 percent excess, based on polyunsaturation in the oil); sulfur dioxide (0.5 percent, based on oil); and hydroquinone (0.67 percent, based on oil) are mixed in an autoclave and heated to 290° C. for 1 hour. The unreacted dimethyl maleate is stripped off at about 200° C. and 0.1 mm. absolute pressure. The residual sulfur dioxide is removed by washing or similar means. The adducted oil is analyzed by transesterification and distillation of the resultant esters.

Properties: The adducted oils are viscous amber liquids. Typical properties of a dimethyl maleate adduct of soybean oil are:

Refractive Index	$n^{30/D}$ 1.4806
Iodine Value	47.8
Acid Value	17.2
Yield of adduct, based on linoleate plus linolenate	98%
Undistillable residue	13%

Use as Plasticizer for Nitrile Rubbers: Evaluation data for the dimethyl maleate adduct of safflower oil in a standard 30 PHR softener study recipe with Hycar 1001* are given in Table I. This adduct showed excellent compatibility with this nitrile

* This material is named merely as part of the exact experimental conditions. Naming it does not constitute an endorsement of this product over those of other manufacturers.

rubber with no bleeding noted from the mixed stock or vulcanizate originally or after 1 month's storage. Further studies have shown that the adducts of soybean and linseed oils have essentially equivalent utility in the plasticizing nitrile rubbers.

REFERENCES

1. Reactions of Dienophiles with Vegetable Oils. I. Reactions of Maleic Esters with Sulfur Dioxide Catalyst. Miller, W. R., Bell, E. W., Cowan, J. C., and Teeter, H. M. J. Am. Oil Chemists' Soc. 36(9): 394-397 (1959).

2. Safflower Oil Adducts as Plasticizers. Teeter, H. M., Cowan, J. C., Gast, L. E., Yurgen, W. J.,[1] and Clark, R. A.,[1] ([1]Battelle Memorial Institute, Columbus, Ohio). J. Am. Oil Chemists' Soc. 38(3): 117-120 (1961).

3. Pressure Reaction of Maleic Esters with Vegetable Oils. Miller, W. R., Bell, E. W., Cowan, J. C., and Teeter, H. M. J. Am. Oil Chemists' Soc. 38(5): 235-237 (1961).

Reprints may be obtained for these references from the Northern Utilization Research and Development Division, U. S. Department of Agriculture, Agricultural Research Service, Peoria, Illinois.

Table I

Evaluation of Dimethyl Maleate-Safflower Oil Adduct as Nitrile Rubber Plasticizer[a]

Plasticizer	Safflower oil adduct	Dioctyl phthalate	Polymeric	None
Milling Appearance	Good Smooth and glossy	Good Smooth and glossy	Very good Smooth and glossy	-- Slightly rough, semi-glossy
Mooney scorch time at 270° F. (min.)	20	16-1/2	--	--
Modulus at 300% elongation (p.s.i.)	1080	1330	1580	2760
Ultimate tensile strength (p.s.i.)	2220	2740	2030	3060
Ultimate elongation (%)	650	600	430	360
Hardness (Duro A)	63	53	65	75
Compression set--ASTM Method B--70 hr. at 212° F. (%)[b]	68	68	67	66
Low temperature brittleness-ASTM D746				
Pass (°F.)	-15	-40	-5	-5
Fail (°F.)	-20	-45	-10	-10
Immersions				
Air oven--70 hr. at 300° F.				
Hardness change (points)	+20	+39	--	--
Weight change (%)	-2.5	-16.3	-1	--
ASTM Oil No. 1--70 hr. at 212° F.				
Tensile change (%)	+7	+7	+14	+6
Elongation change (%)	-29	-43	-23	-17
Hardness change (points)	+4	+21	+5	+5
Volume change (%)	-5.6	-19.0	-2	-2
ASTM Oil No. 3--70 hr. at 212° F.				
Tensile change (%)	-2	+9	-2	+10
Elongation change (%)	-26	-28	-23	+3
Hardness change (points)	-6	+12	-1	-2
Volume change (%)	+9.2	-10.6	+9	+7

[a] Test specimens cured at 310° F. for 30 minutes, except as noted.

[b] Block cured 45 minutes at 310° F.

CA-N-20
February 1962

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

GUMS AND MUCILAGES

CROTALARIA INTERMEDIA GUM, A HIGH VISCOSITY PRODUCT

This product is available as a result of the cooperative efforts of USDA chemists, botanists, and engineers who are investigating numerous uncultivated plant species with the objective of developing new crops.

Nature and Origin: Crotalaria gum (or mucilage) is obtained from seeds of several species of the genus *Crotalaria*. The species described here, *C. intermedia*, is a heavy-seeding annual of the legume family. Further botanical or agronomic information about it may be obtained from New Crops Research Branch, Crops Research Division, Agricultural Research Center, Beltsville, Maryland.

The gum occurs in the endosperm of the mature seed. The gum is a high molecular weight galactomannan, related chemically to guar and locust bean gums. The ratio of mannose to galactose in crotalaria gum is near 3; in guar gum, 2; in locust bean gum, 4.

Preparation: The gum is prepared from whole seeds by a dry-milling process which involves either fine grinding and screening or grinding and air classifying. The embryo and hull portions of the seed are more easily ground than the endosperm or gum portion. The endosperm portion is separated from the other seed parts and reground to aid dispersion. The reground material constitutes the final gum product. It is treated to stabilize and enhance the viscosity of aqueous dispersions.

Physical Properties:

Solid.--Off-white powder which passes a 100-mesh screen and contains about 10 percent moisture at usual relative humidity. Analysis (dry basis): protein, 4.5 percent; residue insoluble in approximately 0.4 *N* H_2SO_4 at 100° C. for 6 hours, 3.2 percent.

Solution.--Easily dispersed in cold or hot water to form a nearly colorless opalescent solution. The unadjusted pH of a 1.0-percent solution is 6.5 to 6.7.

Viscosity Relationships*: *C. intermedia* gum may be dispersed in either cold or hot water to form stable, viscous solutions. Suspension of the mucilage in hot water yields a higher viscosity than does suspension in cold water (Figure 1). The gum solutions were agitated 5 minutes at the indicated temperatures and allowed to cool to 25° C. before measurement. The viscosity-concentration curve is shown in Figure 2.

* All viscosity data were obtained with a Brookfield LVT viscometer. Mention of an apparatus by name does not constitute endorsement by the United States Department of Agriculture over similar instruments manufactured by others.

The plot of viscosity vs. rate of shear shows shear-thinning properties (Figure 3).

The viscosity of solutions decreases with increasing temperatures (Figure 4).

C. intermedia gum solutions are stable from pH 5 to pH 9 (Figure 5). At pH 3 or pH 11 the solutions show a slight loss in viscosity on aging (pH adjusted with HCl or NaOH).

Added salt moderately lowers the viscosity of a 1.0-percent gum solution (Figure 6). A 1.0-percent gum solutuon is gelled by the addition of 100 mg. borax per 100 ml. of solution. As little as 10 mg. borax will form a soft gel (viscosity approximately 25,000 centipoise) if the pH is adjusted to be slightly alkaline.

Applicability: *C. intermedia* gum may be useful where a dispersion or thickening agent is desired. Laboratory experiments indicate its potential as a superior material for use as a wet-end additive to increase strength properties of papers (1).

The germ fraction of the seed is toxic to rats, as is the whole seed. However, preliminary feeding tests of the gum fraction described here indicate no short-term toxicity for rats other than retarded growth. Chronic toxicity studies on the mucilage and more quantitative comparisons of toxicity of this species with others in the *Crotalaria* genus are incomplete.

Reference:

1. Evaluation of Seed Galactomannans from Legumes as Paper Sizes.
H. L. Tookey, A. J. Ernst, R. L. Lohmar, and I. A. Wolff.
TAPPI 44(12), 910 (1961).

Fig. 1. Viscosity of 1.0% Crotalaria Gum versus Time

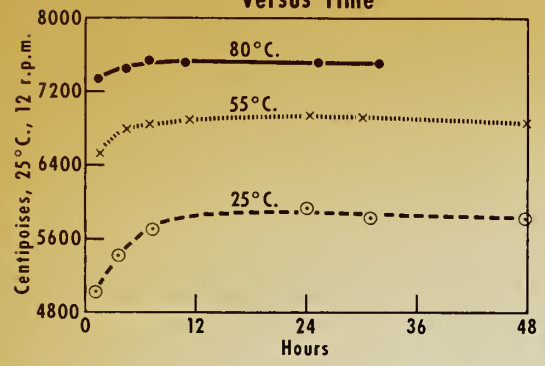


Fig. 2. Viscosity* versus Concentration of Crotalaria Gum

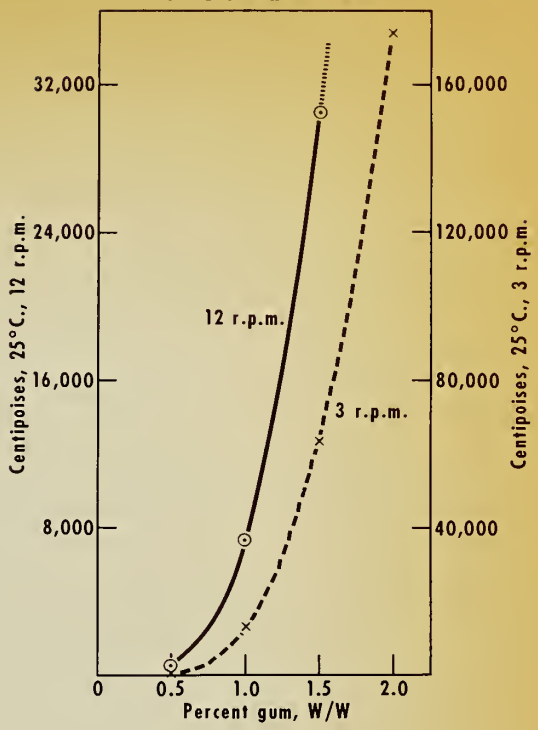


Fig. 3. Viscosity* of 1.5% Crotalaria Gum versus Rate of Shear

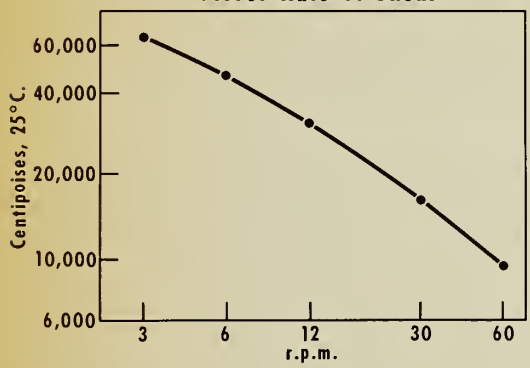


Fig. 4. Viscosity* of 1.0% Crotalaria Gum versus Temperature of Solution

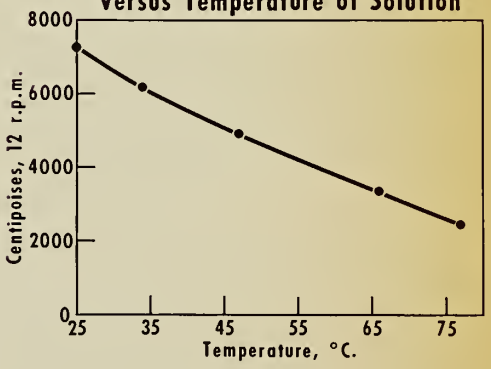


Fig. 5. Viscosity of 1.0% Crotalaria Gum versus pH

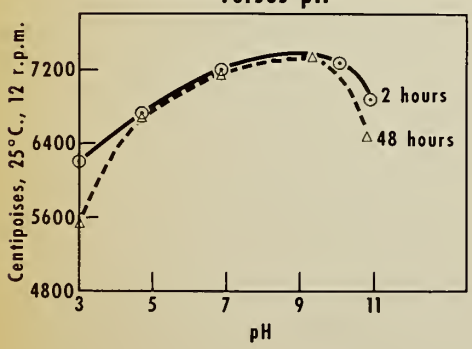
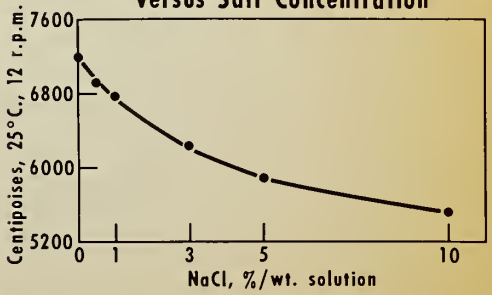


Fig. 6. Viscosity* of 1.0% Crotalaria Gum versus Salt Concentration



* Gum was suspended at 80°C.; viscosity measured after 16-20 hours

CA-N-21
May 1962

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

MICROBIAL POLYSACCHARIDES

Information on Polysaccharide B-1973

This product is one of a series of polysaccharides made by fermentation processes developed at this Division.*

Information presented here is preliminary and is subject to further confirmation and extension as current research progresses and as industrial evaluation justifies.

Nature and Origin: An extracellular, high-molecular-weight heteropolysaccharide produced by the bacterium Arthrobacter sp. NRRL B-1973.

Preparation: Fermentation of a medium containing 2 to 4 percent commercial dextrose, organic nitrogen, dibasic potassium phosphate, and manganese and magnesium ions. Incubation is for 4 days at 25° C. with moderate aeration and agitation; final viscosity of culture, 10,000 to 12,000 centipoises.**

Purification and Isolation: Centrifugation to remove cells; precipitation by methanol in the presence of electrolyte such as potassium chloride; reprecipitations; and finally dehydration of the gelatinous, somewhat fibrous gum by methanol. Other methods of purification are under investigation. Yields of purified product under experimental conditions to date have been about 45 percent based on D-glucose.

Composition: Polysaccharide B-1973 contains D-galactose, D-glucose, and mannuronic acid in the estimated molar ratio of 0.75:0.75:1.00. The mannuronic acid content in laboratory-purified product is about 28.5 percent; the acetyl content, which is present as the O-ester, is about 25 percent. Nitrogen and sulfated ash contents of laboratory-purified product are 0.07 percent and 12.5 percent, respectively; corresponding values for the pilot-plant product are about 0.75 percent and 20.6 percent.

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* Information on Phosphomannan Y-2448, CA-N-7, October 1958.
Information on Polysaccharide B-1459, CA-N-9, September 1959.
Information on Phosphomonoesters of Mannose Polymers, CA-N-11, May 1960.
Information on Polysaccharide Y-1401, CA-N-14, April 1961.

** All viscosity data were obtained at 25° C. with a Brookfield viscometer operating at 30 r.p.m. Under these conditions the maximum viscosity that can be measured is about 20,000 cps. This equipment is named merely as part of the exact experimental conditions. Naming it does not constitute an endorsement of this apparatus over those of other manufacturers.

Properties of Native Polysaccharide:

Gum.--Gelatinous, somewhat fibrous, somewhat cohesive.

Solid.--As now prepared on a pilot-plant scale, the solid is light tan in color and consists of soft, slightly fibrous particles. When equilibrated under conditions of 50 percent relative humidity at 20° C., the solid contains about 14 percent moisture. Particles swell and disperse at essentially the same rate in either cold water or 5 percent potassium chloride solution, and the final viscosity is the same in both solvents. If a suspension of solid in either of these solvents is stirred continuously, a steady viscosity value is reached within a half hour.

Aqueous Solutions.--Residual bacterial cells impart opalescence. Solutions appear to be dilatant, and show plastic rheological characteristics and yield values. The pH is in the range 7 to 8 for concentrations of 0.5 to 2.0 percent. When made to 35 percent concentration in ethanol, aqueous dispersions remain homogeneous but increase 20 percent in viscosity.

Specific Rotation.-- -11° (c 0.25, in water after autoclaving). Unreliable values are obtained on solutions prepared in cold water, in dilute salt, or in 35 percent ethanol.

Modified Forms of Polysaccharide:

Autoclaved Native.--Steam autoclaving aqueous solutions of native polysaccharide at 15 lbs./in.^{2} pressure (121° C.) for suitable times improves some properties, changes others, but does not affect still others of value. Viscosity is increased, especially for concentrations 1 percent and greater; some physical organization appears to be disrupted, as indicated by observations on specific rotation; the solutions become more cohesive, show "longer" flow and stronger gel properties which are enhanced by aging and cooling; solutions are pseudoplastic and dilatant.

Deacetylated.--Acetyl groups are removed rapidly by dilute alkali in the presence of potassium chloride at room temperature under oxygen-free conditions. A 35-percent concentration of methanol precipitates a fibrous product; twice this concentration is required to precipitate the native polysaccharide. After isolation, the dry deacetylated product disperses readily in cold water to form pseudoplastic solutions. The deacetylated polysaccharide in solution requires greater force to produce shearing action, and is less shear-sensitive than is the native.

Viscosity Relationships:** Viscosity-concentration curves for the native and two modified forms of polysaccharide B-1973 are shown in Figure 1. For comparison, curves are included on commercial-grade, high-viscosity sodium alginate and on our microbial polysaccharide B-1459.

The effects of increasing salt concentrations up to 5 percent in aqueous solutions of laboratory-purified native and deacetylated polysaccharides are shown in Figure 2. For 1 percent native polysaccharide, little further change in viscosity and no insolubilization results from saturation with potassium chloride or from 20 percent calcium chloride. However 20 percent concentrations of sulfates of mono- and divalent cations precipitate both the native and deacetylated polysaccharides. The native does not complex with borax; the deacetylated forms cohesive gels when concentrations of borax or of potassium chloride exceed about 2 percent.

Solutions of typical polyanions (1 percent) show 30 to 90 percent decrease in viscosity in the presence of 1 percent potassium chloride.

Solutions of native polysaccharide B-1973 (0.1 percent and 1.0 percent in water) show about 5 percent decrease in viscosity when heated to 100° C. and then cooled, but the deacetylated shows about 25 percent decrease. Heating the native (1.0 percent) in potassium chloride (5 percent) does not change the viscosity.

The viscosity of native polysaccharide is essentially constant between pH 4 and 11 (Figure 3). Deacetylation accounts for the decrease in viscosity of solutions initially at pH 12 and 13, and also for the decrease with time of pH's initially greater than 7.

Properties of Unsupported Films: Solutions of native polysaccharide (1 percent) do not cohere sufficiently to form a film. Solutions of the native polysaccharide, autoclaved under suitable conditions, produce films of very good tensile strength and excellent flexibility, even when unplasticized. Deacetylated polysaccharide gives excellent films either plasticized or unplasticized.

Applicability: Application will be favored by the properties presented by the three forms in which this polysaccharide can be obtained. Each form has certain advantages.

Native.--Ease of dispersion in cold water or salt solutions; high solution viscosity in water alone as well as in presence of salts or 35 percent ethanol; heat-stable viscosity in water or in salt solution.

Autoclaved Native.--Increased viscosity and cohesiveness of solutions, and greatly improved properties of films (unsupported, even unplasticized) as compared with those of the native polysaccharide.

Deacetylated.--Low-alcohol concentration required to precipitate from aqueous solution; ease of dispersion of solid in water; high solution viscosity which is stable to salts; strength and flexibility of unsupported films.

Fig. 1
Viscosity-Concentration Curves
Polysocchoride B-1973 Compored with Other Polysocolloids

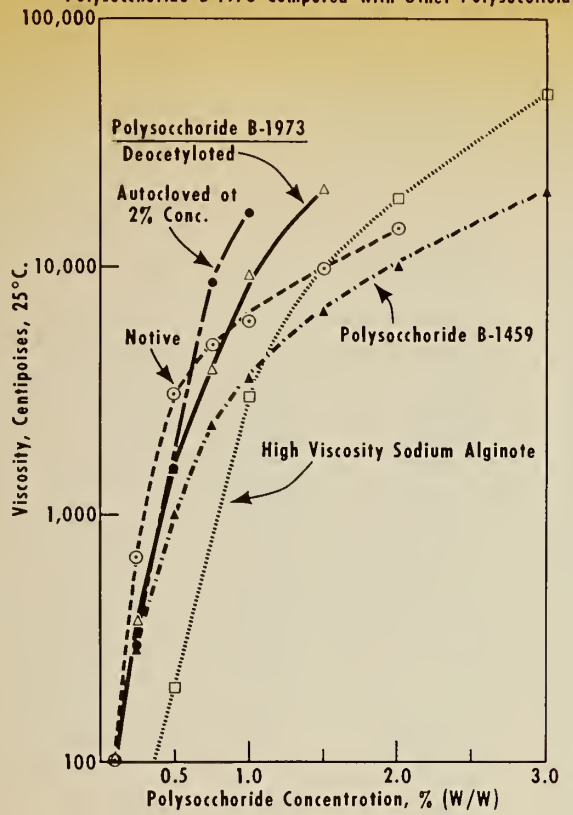


Fig. 2
Polysaccharide B-1973
Viscosity of Notive ond Deocetyloted in Presence of Solts

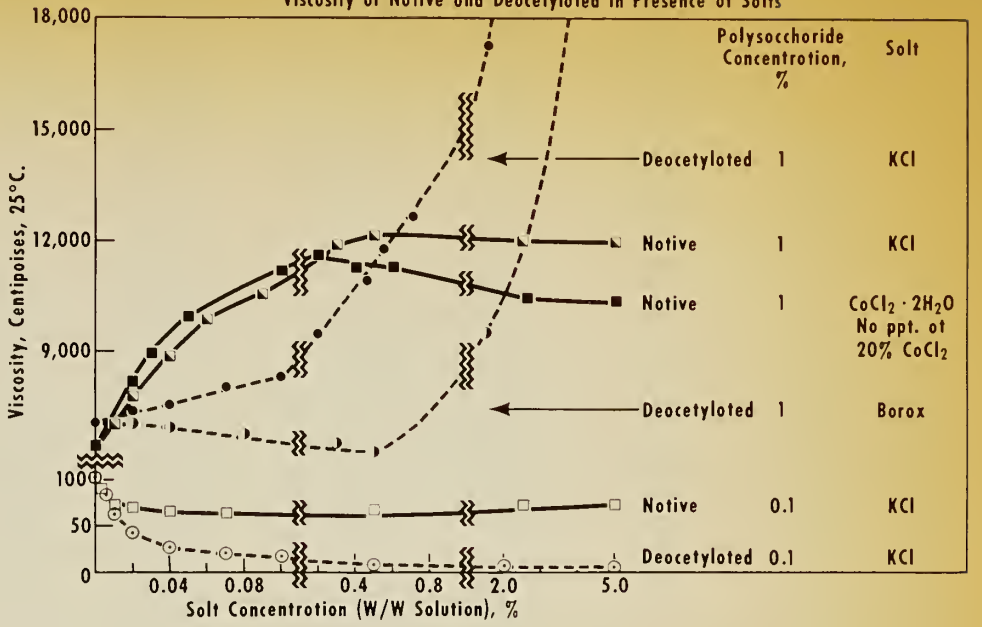
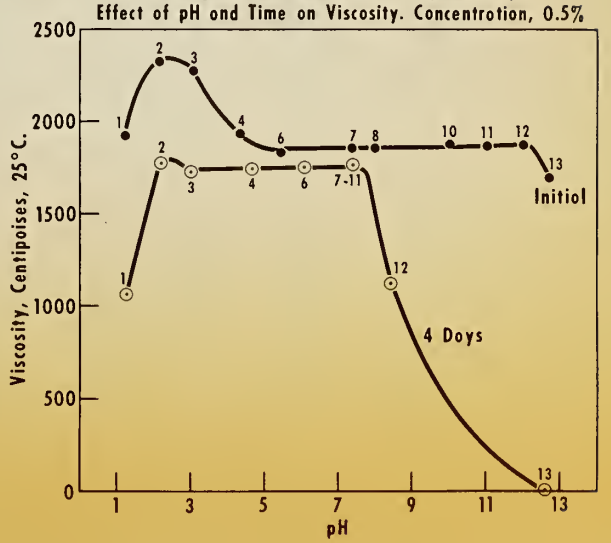


Fig. 3
Polysaccharide B-1973 (Native)
Effect of pH ond Time on Viscosity. Concentrotion, 0.5%



CA-N-22
July 1962

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

Northern Utilization Research and Development Division
Peoria, Illinois

CEREAL PRODUCTS FOR THE PAPER INDUSTRY

Cereal Pulps in Papermaking

A simple method for preparing xanthated products from various cereal grain materials and utilizing these novel cereal derivatives in papermaking has been developed in the laboratory.

Experimental papers formed from blends of crosslinked cereal xanthates--termed "cereal pulps"--and wood pulp exhibit significant improvements in several strength properties. Other beneficial effects from use of cereal xanthate derivatives have also been noted in some pulps and papers prepared in this study.

Different types of wheat and corn products--ground whole grain, flour, bran, and starch--were studied as raw materials for making cereal pulps. Low-cost reagent chemicals only are required for the procedure and chemical reactions involved are quickly and easily carried out.

While some scope of the possibilities for using cereal pulps in papermaking is demonstrated in this exploratory investigation, no application in a specific type of paper has been thoroughly examined. Studies of effects of the degree of xanthate substitution, level of addition, crosslinking agent, and processing conditions on quality and utility of different cereal pulps are in progress.

This research on application of a new group of cereal derivatives in production of paper was reported (1) at the New York meeting of the Technical Association of the Pulp and Paper Industry, February 1962. Reprints of the article, following publication in TAPPI, will be available at:

Northern Utilization Research and Development Division
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Peoria, Illinois

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INVESTIGATION OF CEREAL PULPS

Xanthates of starch and other cereal-derived products have not been used heretofore for producing papers. Exploratory studies here reported were to survey possible applications of crosslinked cereal xanthates in paper.

Important to use of cereal xanthates in papermaking is their ability to be rapidly converted into the insoluble cereal pulps while in an intimate blend with a dilute suspension of wood pulp. Cereal pulps precipitate on wood pulp fibers to become incorporated as an integral part of the paper product.

In exploring possible applications of different cereal pulps in papermaking, the corresponding cereal xanthates were crosslinked in the presence of representative commercial wood pulps. Physical properties of experimental papers produced from such blends indicate the suitability of cereal pulps for making a variety of paper types.

TRIAL APPLICATIONS OF CEREAL PULPS

Application of cereal pulps in paper production involves two main steps. A water-soluble cereal xanthate is produced from the starting cereal grain product. The xanthate intermediate in aqueous solution is then blended with the wood pulp furnish and crosslinked *in situ* prior to paper formation.

Data on preparation and properties of experimental papers (Table I) made in this investigation from several combinations of cereal pulps and wood pulps were derived largely from handsheets. Except for pH control, handsheets were prepared according to TAPPI standard procedures. Several types of paper containing cereal pulps were made on a laboratory-scale fourdrinier machine. Aside from the xanthate intermediates, no other additive was used in the test pulps or papers.

Density, opacity, and dry-strength properties of all test papers in studies covered by this report were determined by TAPPI standard procedures. Wet-tensile strength was similarly determined, with 30 minutes of soaking, at 23°C., before testing.

Preparation and Testing of Kraft-Type Experimental Papers: Studies of general properties of papers produced from cereal pulp-wood pulp furnishes were made with softwood sulfate (kraft) pulps (S.R.700).[1/]

Xanthated cereal intermediates used in kraft-type papers included those of wheat starch, corn amylose (92 percent), ground whole wheat, ground wheat bran, and canary dextrin. Some finished experimental sheets contained as much as 44 percent cereal pulp.

1/ Schopper-Reigler freeness value in ml.

Cereal xanthates with various degrees of substitution (D.S.) were added to wood pulp at different addition levels to determine feasible ranges for these and other variables.

For comparison, a few tests were made with xanthated derivatives of cellulose (bleached sulfite), guar gum, polyvinyl alcohol, dextran, and sucrose as intermediates.

Tests made on handsheets produced in these preliminary experiments indicated that:

--Method used for incorporating cereal pulp in paper is applicable to a wide variety of xanthated cereal derivatives.

--Retention in the finished sheet with crosslinked xanthated cereal materials under most conditions was high; also, the percent of added cereal pulp which is retained increased at higher levels of addition.

--Retention and strength properties differed little between amylose cereal pulp and whole-starch cereal pulp.

--Degree of xanthate substitution did not appear to have a marked effect on amount of xanthide cereal pulp retained in the paper.

--Opacity decreased and resistance of the sheets to water penetration increased generally as content of cereal pulp was increased.

--Strength properties of the sheets, except for tear, tended to increase as cereal pulp content of the paper increased to about 20 percent, and then decreased. In many instances, however, burst and dry-tensile strengths were significantly improved at higher cereal pulp levels. Tear strength usually decreased significantly when cereal pulp content of the paper exceeded about 5 to 10 percent.

--Wet-strength values were particularly high for most test sheets.

--Xanthate D. S. appeared to influence strength properties; the apparent optimum D. S. for maximum strength is around 0.16.

--Use of chlorine for the crosslinking step gave cereal pulps equal to or superior to those produced by the other oxidants tested in improving strength properties of papers.

--During a year's storage in the laboratory, handsheets with 1 to 44 percent starch xanthide (as the cereal pulp) showed no significant changes in strength or other evidence of deterioration.

Experimental Machine Paper: A few experimental papers were made on a 10-inch fourdrinier from various cereal pulp-wood pulp combinations to determine how the presence of cereal pulps would affect continuous operation. Xanthates of wheat starch, ground whole wheat, and wheat bran were each added to wood pulp at the chest and each crosslinked with either zinc ions or iodine.

The mixed furnishes ran as well as the wood pulp controls. No sulfur odors were detected at the drying rolls nor other evidence of xanthide decomposition noted.

For all cereal pulps tested, wet-tensile strength of the finished sheet was considerably improved over that of the control. The wet strength of paper obtained with starch xanthide may be considered to be of the permanent type; the wet-tensile value was not significantly changed when an experimental sheet was soaked for 24 hours. Dry-strength properties of the machine sheets generally were not greatly changed by any of the cereal pulps.

Application in Newsprint: Increases in dry- and wet-strength values obtained for experimental papers by cereal pulp addition indicate possible application of cereal pulps in newsprint to replace the long-fiber pulp.

An experiment to determine suitability of groundwood-cereal pulp blends for newsprint production showed (Table I) that 4 percent of cereal pulp in the pulp blend was essentially equivalent to 16 percent of long-fiber pulp for improving dry-strength properties. The tear value, however, was lower. Wet strength of this experimental sheet was ten times that of newsprint containing long-fiber pulp; opacity of the sheet was about the same. Pulp drainage time was unaffected by addition of cereal pulp.

Application in Greaseproof-Type Paper: Because high-level incorporation of cereal pulps had produced significantly lower opacity in some experimental nongreaseproof-type papers containing wood pulps prepared with the usual beating time (pulps for greaseproof papers, of course, require longer beating), and because freeness of even some relatively fast-draining pulps was observed to be improved by cereal pulp addition, studies were made to determine whether cereal pulps would help speed up these two production factors for greaseproof paper.

Partially hydrated, bleached sulfite pulp (S.R. 500) was tested to see whether the poor transparency of paper made from it, due to insufficient beating, could be offset by adding cereal pulp to the furnish. A more highly hydrated, bleached sulfite pulp (S.R. 250) also was tested to determine whether drainage time could be improved by cereal pulp.

Results of these trials clearly demonstrated that, under the conditions employed, cereal pulp reduced opacity of greaseproof papers considerably and decreased very substantially the drainage time of pulps from which they were made (Table I). These experimental papers that contained cereal pulps also showed high resistance to water and oil, and reacted positively in the blister test. Wet-strength value in each case was more than double that of the control.

Based on these and other tests, the use of cereal pulps could permit a more rapid production of greaseproof papers from less highly beaten wood pulps.

PREPARATION AND PROPERTIES OF CEREAL XANTHATES AND XANTHIDES AS RELATED TO USE AS CEREAL PULPS

As outlined in the preceding section, formation of cereal pulps is a two-step process. First, the cereal grain raw material is converted to the water-soluble cereal xanthate. Next, the xanthate derivative in aqueous solution is converted to the insoluble xanthide by formation of crosslinks between molecules of the original cereal xanthate. Combination of cereal xanthide (as cereal pulp) with wood pulp in papermaking has already been discussed.

Although any one of several types of reactions will readily crosslink cereal xanthates to give the insoluble derivatives for the second step, reactions used in this study were oxidative crosslinking (5,6) which forms the cereal xanthides; and crosslinking by heavy metal ions (7), which produces the insoluble metal cereal xanthates or mixtures of the xanthide and metal xanthate (Figure 1).

Cereal Xanthates--Preparation: The xanthation reaction is applicable to many substances, including natural polymeric materials such as cellulose, starch and protein that contain hydroxyl (Figure 1), amino, or thiol groups. Xanthation of starch and most cereal products is a much simpler procedure, however, than the familiar process of xanthating cellulose in the manufacture of viscose rayon. Gelatinization of starch with alkali occurs more readily, and production of a soluble starch xanthate need not involve pretreatment of starch with alkali and aging as is the case with cellulose.

A simple laboratory procedure was used in preparing xanthates of starch and other cereal products for testing in paper applications (1-4). Wheat starch was reacted with low-cost reagents, sodium hydroxide and carbon disulfide, to prepare sodium starch xanthate (Figure 1). Various proportions of the reactants in differing solution concentrations were added to the starch at room temperature to obtain the xanthate D.S. desired.

By the same general procedure wheat flour, ground whole wheat, wheat bran, wheat gluten, canary dextrin, and corn amylose (92 percent) were readily converted to their respective xanthates.

Although further laboratory studies are required to develop reaction conditions that will produce cereal xanthates in highest yields, preliminary experiments have indicated that machine-mixing procedures, employing a continuous-type operation, may permit their economic production as cereal pulp intermediates.

Cereal Xanthates--Properties: A combination of several properties enables cereal xanthates to function as useful intermediates in the formation of cereal pulps for paper production. Cereal xanthates are soluble in water. In solution, their chemically reactive xanthate groups (thiolthiocarbonyl) permit them to be quickly converted to insoluble derivatives by any of a number of crosslinking agents, including several low-cost reagents that are easily applied at the wet end of the papermaking process. Furthermore,

the crosslinking reaction is such that the cereal xanthates are substantially removed from dilute solutions, even when the xanthate D.S. is quite low. The water-soluble nature of the xanthate thus permits the insoluble xanthide to be formed and applied in situ to wood pulp furnishes.

Cereal Xanthides--Preparation: Cereal xanthides have been obtained in good yields in the laboratory (1) by crosslinking cereal xanthates in dilute aqueous solutions (2 percent or less) with an inexpensive oxidizing agent (Figure 1). The reaction was carried out in a neutral solution carefully maintained at or near pH 7 during slow addition of the oxidant. When oxidation was completed, the xanthides were removed by filtration; the product was washed first with water, then with alcohol; and dried at 75° C. in vacuum.

Xanthates of wheat starch, soft wheat flour, ground whole wheat, ground wheat bran, wheat gluten, canary dextrin, and corn amylose (92 percent) were readily converted to the corresponding xanthides.

Iodine, chlorine, nitrogen tetroxide, and nitrous acid were the oxidants employed. Although iodine was used in most of the exploratory experiments, chlorine would be preferred costwise in practical crosslinking applications.

Some loss of sulfur was noted in the crosslinking procedure; this loss varied with the oxidant used, its rate of addition, and the precision of pH control.

Xanthation of wheat gluten and subsequent oxidative crosslinking to produce the insoluble protein xanthide was accomplished with good yield and without loss of nitrogen. Gluten in wheat flour thus is derivatized like the other main flour component, starch.

Cereal Xanthides--Properties: Cereal xanthides are extremely high-molecular-weight polymers having properties different from the natural polymers that make up their cereal starting materials. Xanthides generally are hydrophilic but are nondispersible in water and do not form sols or gels.

Properties of cereal xanthides that permit their application as cereal pulps in papermaking are (1) their formation and precipitation from dilute cereal xanthate solutions and (2) their stability toward heat and a number of reagents.

Considerable information on stability of cereal xanthides was developed which indicates their stability may be adequate for use in papermaking. Xanthides with as few as one crosslink per 120 anhydroglucose units are insoluble in boiling water. They are also insoluble in boiling 5-percent acetic acid and in boiling 1 molar sodium sulfite. Even in 3-percent aqueous sodium hydroxide their rate of swelling and dissolution is slow. Treatment of several cereal xanthides with strong acids (pH 2) at room temperature for an hour is without effect on their property of swelling. In drying wet, swollen starch xanthide (7 hours at 100° C. in vacuo), a slight loss of sulfur is noted during the first hour only; this is believed

to come from unreacted xanthate groups present. Mention was made earlier that no odor at the drying rolls nor other evidence of xanthide decomposition is noted in fourdrinier production of paper containing cereal pulps.

Heavy Metal Xanthates: Certain heavy metal cereal xanthates are insoluble in water. Such insoluble xanthates can be incorporated in paper by adding the metal salt to the cereal xanthate in the presence of wood pulp.

Cupric and ferric ions were reacted with wheat starch xanthate to give the corresponding metal starch xanthates and starch xanthide (Figure 1). Zinc ions react with starch xanthate to form an insoluble zinc starch xanthate (7).

The dried zinc starch xanthate is white but the corresponding copper and iron products are dark colored. As a consequence of their color, the copper and iron products have limited potential as cereal pulps in paper products.

Heavy metal xanthates appear to be sufficiently stable for use in paper as cereal pulps.

Literature Cited

1. Russell, C. R., Buchanan, R. A., Rist, C. E., Hofreiter, B. T., and Ernst, A. J., "Cereal Pulps. I. Preparation and Application of Crosslinked Cereal Xanthates in Paper Products," preprint of paper presented at the 47th Annual Meeting of the Technical Association of the Pulp and Paper Industry in New York, N. Y., February 18-22, 1962.

2. Cross, C. F., Bevan, E. J., and Briggs, J. F., J. CHEM. SOC. 91: 612-614 (April 1907).

3. Ost, H., Westhoff, F., and Gessner, L., ANN. CHEM. LIEBIGS 382: 340-360 (April 1911).

4. Adamek, E. G., and Purves, C. B., CAN. J. CHEM. 35: 960-968 (September 1957).

5. Cambron, A., and Whitby, G. S., CAN. J. RES. 2: 144-152 (January 1930).

6. Harrison, W., U. S. Pat. 1,680,020 (August 7, 1928).

7. Little, L. H., Poling, G. W., and Leja, J., CAN. J. CHEM. 39: 745-754 (April 1961).

Table I.--Properties of Papers Made with Cereal Pulp-Wood Pulp Furnishes[a]

Pulps Used	Cereal Pulp: Level added, g.[b]	Cereal Pulp: RU/CL[c]	Pulp drain time, sec.	Retention in O.D. sheet[d], %	Paper Properties[e]: Burst	Tear	Breaking length, M.: Dry	Breaking length, M.: Wet	Schop. fold: double	Other[f]
Kraft[g], unbleached										
Pulp #1 - control	none	--	--	--	59	137	8,680	290	1,450	D - 0.64
1	50[h]	18	--	28	56	73	7,960	1,090	870	D - 0.78
Pulp #2 - control	none	--	--	--	54	153	7,600	220	1,260	-- --
2	4	9.6	--	1.6[i]	57	146	8,520	770	1,130	-- --
2	40	9.6	--	18.7[i]	77	82	9,500	1,600	1,510	-- --
2	80	9.6	--	40.4[i]	68	62	8,520	1,570	1,080	-- --
Pulp #3 - control	none	--	--	--[j]	43	115	9,480	350	1,420	-- --
3	50	--	--	13.2[i,j]	48	61	9,000	2,260[k]	730	-- --
Kraft[g], bleached										
Pulp #4 - control	none	--	--	--	62	155	8,630	230	1,170	-- --
4	20	17	--	10	75	94	10,030	1,090	1,460	-- --
4	80	17	--	40	59	59	8,110	940	1,120	-- --
Pulp #5 - control	none	--	5	--	57	152	8,030	230	1,020	O - 68; DT - 115
5	50[h]	40	5	19	71	72	9,710	2,090	2,900	O - 45; DT - 600+
Pulp #6 - control	none	--	--	--[j]	59	119	11,800	300	1,450	-- --
6	10[l]	--	--	2,3[i,j]	58	100	11,720	1,030	1,520	-- --
Groundwood (for newsprint)										
Pulp #7 - control	none	--	24	--	12	56	3,220	50	--	BW - 60; D - 0.43; O - 98
7 pulp (84%) plus kraft, unbl. (16%)	none	--	24	--	18	74	3,740	60	--	BW - 59; D - 0.45; O - 96
7	4	--	24	--	16	40	3,750	600	--	BW - 60; D - 0.47; O - 94
Sulfite, bleached (greaseproof paper stock)										
Pulp #8 (SR 250)-cont.	none	--	107	--	37	29	7,700	370	190	O - 46
8 (SR 250)	40[m]	1.67	36	29	39	23	7,600	750	140	O - 30
Pulp #9 (SR 500)-cont.	none	--	52	--	62	51	9,670	530	2,470	O - 48
9 (SR 500)	50[h,n]	59.	30	23	63	45	9,070	1,210	4,510	O - 30

Footnotes

Footnotes

a/ Cereal pulp was formed in wood pulp furnish by addition of wheat starch xanthate solution and subsequent precipitation (as starch xanthide) by crosslinking with I_2, except as noted.

b/ Given as grams of oven dried starch used in preparing the starch xanthate intermediate, per 100 grams of oven dried wood pulp.

c/ Repeating units per xanthide (or metal ion) crosslink.

d/ Determined by subtracting weight of wood pulp used from weight of sheet, except as noted.

e/ All data from handsheets, except as noted (j/).

f/ BW - basis weight; D - density, g./cc.; O - opacity, %; DT - drop test, time in seconds required for water drop to be absorbed.

g/ Softwood sulfate, Schopper-Riegler pulp freeness value (SR)--700 ml.

h/ Cl_2 used as oxidant for crosslinking.

i/ Determined by sulfur analysis of paper product.

j/ Paper produced on 10-inch fourdrinier machine; tear, dry strength, wet strength, and fold values tested in machine direction.

k/ Wet strength after 24 hours' soaking was 2,180.

l/ $ZnCl_2$ used as source of metal ion for crosslinking.

m/ Wood pulp consistency during crosslinking was 1.5.

n/ Wood pulp consistency during crosslinking was 0.375.

Fig. 1--Reactions Involved

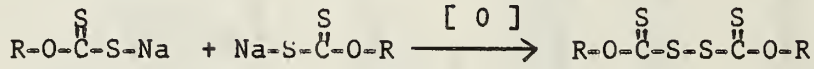
XANTHATION

Starch

Sodium starch xanthate

CROSSLINKING

Oxidative



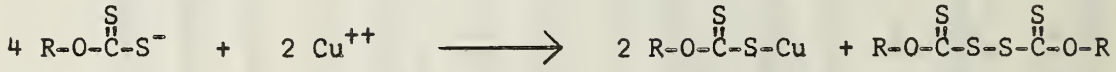
Sodium starch xanthate

Starch xanthide (Starch disulfurdicarbothionate)

Heavy Metal Ion

Starch xanthate radical

Zinc starch dixanthate (Zinc dithiocarbonate of starch)



Starch xanthate radical

Cuprous starch xanthate

Starch xanthide

CA-N-23
August 1963

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

NEW SEED OILS

Crambe Seed Oil (High Erucic Acid Content)

For several years new vegetable oils have been sought that might be produced domestically and be useful to industry. Selection has been based on presence in the oil in substantial percentage of fatty acid(s) not derivable from other oilseeds grown in this country. Crambe oil is a promising selection that is now under intensive investigation at the Northern Division. The oil has been isolated by solvent extraction of crambe seed grown under auspices of the U. S. Department of Agriculture and state agencies.

Nature of the Oil: Crambe oil is a glyceride oil that contains 55-60 percent of esterified erucic acid as determined by gas-liquid chromatographic analysis of methyl esters derived from the oil. Erucic acid is a 22-carbon monobasic acid containing one cis olefinic bond between carbons 13 and 14 as follows:

$$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$$
cis

Crambe oil resembles rapeseed oil in composition, but contains more erucic acid than rape varieties currently grown commercially in other countries.

The principal fatty acids in the crambe oil available as samples (degummed, refined, bleached) are erucic (59 percent), linoleic (8 percent), linolenic (7 percent), eicosenoic (4 percent), palmitic (2 percent), and docosanoic (1.5 percent). Other selected physical and chemical constants are recorded below:

Iodine value 91
Gardner viscosity B-C (75 cp.)
Gardner color (2-3); spectrophotometric color 2.6
Free fatty acids 0.4 percent
Unsaponifiables 0.6-1 percent
Density at 25° 0.939 g. per cc.

The iodine value is as expected, based on the fatty acid composition of the oil. Viscosity and density are somewhat greater than for other domestic vegetable oils. Conventional refining and bleaching operations on the crude crambe seed oil gave a good color and a desirably low, free fatty acid content.

Oxidative stability of crambe oil is good because of its large content of monoenoic acid and relatively low iodine value. As determined in an oven at 80°, the induction period was greater than that of both linseed and soybean oils.

Origin: The seed oil is derived from an annual plant, Crambe abyssinica, a member of the mustard family, originally native to the Mediterranean area. Although the species has never been commercially cultivated in the United States, experimental plantings reveal the desirable attributes that suggest easy conversion to crop status if an industrial demand is established. Some of these characteristics are widespread agronomic adaptability, favorable seed yields, amenability to mechanical harvesting with available combines, and indication of good resistance to insect pests and diseases. Requests for information regarding the botanical or agronomic phases of work on crambe should be directed to the New Crops Research Branch, Crops Research Division, U. S. Department of Agriculture, Beltsville, Maryland.

Selected Potential Applications: Erucic acid oils are known to be useful for conversion to rubber additives by crosslinking with sulfur or sulfur compounds. Traditionally, oils of this type have also been useful in lubricants.

Erucic acid derived from crambe oil is convertible by oxidative cleavage to the 13-carbon dibasic acid, brassylic acid, according to the following equation:

$$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH \xrightarrow[\text{Oxidative cleavage}]{\text{Ozone +}} \begin{matrix} HOOC(CH_2)_{11}COOH \\ \text{Brassylic acid} \\ + \\ CH_3(CH_2)_7COOH \\ \text{Pelargonic acid} \end{matrix}$$

Brassylic acid, not now commercially available in quantity, will extend the range of properties attainable with aliphatic dicarboxylic acids in products such as ester plasticizers, ester lubricants, polyamides, and polyesters. A coproduct of cleavage is the 9-carbon monobasic pelargonic acid, a specific industrial chemical.

Suitable research on erucic acid and its derivatives should lead to numerous other expanded applications in the fields of plasticizers, hydrophobic agents, waxlike materials, film-antiblocking agents, and others.

Literature References:

1. Crambe. A Potential New Crop for Industrial and Feed Uses. Crops Res. Div. and North. Util. Res. Devlpmt. Div., Agr. Res. Serv., U. S. Dept. Agr. in cooperation with Iowa, Montana, Nebraska, and Texas Agricultural Experiment Stations. ARS-34-42. September 1962. 9 pp.

2. Utilization Potential of Crambe abyssinica.
Johannes H. Bruun and John R. Matchett.
J. Am. Oil Chemists' Soc. 40(1): 1-5.
January 1963.
Also, Letters to the Editor, J. Am. Oil
Chemists' Soc. 40(4): 142. April 1963.

3. Amino Acid Composition of Seed Meals from Forty-One
Species of Cruciferae.
Roger Wayne Miller, C. H. VanEtten, Clara McGrew,
I. A. Wolff, and Quentin Jones.
J. Agr. Food Chem. 10(5): 426-430, Sept.-Oct. 1962.

4. Amino Acid Composition of Twenty-Seven Selected
Seed Meals.
C. H. VanEtten, R. W. Miller, I. A. Wolff, and
Quentin Jones.
J. Agr. Food Chem. 9(1): 79-82, Jan.-Feb. 1961.

5. Search for New Industrial Oils. V. Oils of Cruciferae
K. L. Mikolajczak, T. K. Miwa, F. R. Earle, I. A. Wolff,
and Quentin Jones.
J. Am. Oil Chemists' Soc. 38(12): 678-681, December 1961.

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

CHRONOLOGICAL ACCOUNT OF THE *STREPTOMYCES ALBUS* PROBLEM AND ITS SOLUTION

The Neotype Strain of *Streptomyces albus* (Rossi Doria) Waksman *et* Henrici

Type Species of The Genus *Streptomyces* Waksman *et* Henrici

The streptomycetes represent a group of microorganisms that are economically important because of their production of antibiotics and other useful compounds. Extending our knowledge about these organisms aids in the development and improvement of fermentation processes, which utilize various agricultural commodities. In streptomycete taxonomy and nomenclature, the most important species is *Streptomyces albus* (Rossi Doria) Waksman *et* Henrici. Although it is the type species of the genus, none of the original cultures are extant. Consequently, it was advisable to select an appropriate neotype strain for *S. albus* and to characterize such a strain more objectively than ever before.

The first four references cited below contain the results of our literature and laboratory studies that led to a proposal for designating a neotype strain. The fifth reference (assembled and attached as a unit) contains the formal proposal and the various actions taken that culminated in official recognition of strain IMRU 3004 (ATCC 3004) as the neotype strain of *S. albus*.

(1) T. G. Pridham and A. J. Lyons, Jr., Bacteriol. Proc.: Abstract G95 (1960).

(2) T. G. Pridham and A. J. Lyons, Jr., J. Bacteriol. 81(3): 431-441 (1961).

(3) A. J. Lyons, Jr. and T. G. Pridham, Bacteriol. Proc.: Abstract G10 (1961).

(4) A. J. Lyons, Jr. and T. G. Pridham, J. Bacteriol. 83(2): 370-380 (1962).

(5) Intern. Bull. Bacteriol. Nomenclature and Taxonomy 12(2): 65; 12(3): 123-126 (1962); 13(1): 23-30; 13(2): 123-124 (1963).

International Bulletin
of
Bacteriological Nomenclature and Taxonomy[1]

Vol. 12, No. 2, p. 65, April 15, 1962.

Proposal for the Recognition of the Neotype Strain of *Streptomyces albus* (Rossi Doria) Waksman and Henrici 1943, p. 339.

REQUEST FOR AN OPINION --- Editorial Board

Lyons and Pridham (1962, 378) have proposed the acceptance of a neotype strain of *Streptomyces albus* (Rossi Doria) Waksman and Henrici 1943, 339 (basionym *Streptotrix* [sic] *alba* Rossi Doria 1891, 399). The proposal is submitted to the Judicial Commission for an OPINION. The reasons for proposal and description of the neotype strain (culture) are detailed in Pridham and Lyons 1961, 431-441 and in Lyons and Pridham 1962, 370-380.

The proposal is that the strain (3004) maintained by the Institute of Microbiology of Rutgers University as IMRU 3004 be approved as the neotype strain of *Streptomyces albus* (Rossi Doria) Waksman and Henrici 1943, 339 (basionym *Streptotrix* [sic] *alba* Rossi Doria 1891, 399). A culture has been deposited with the American Type Culture Collection, Washington, D. C., and is designated as ATCC 3004 (IMRU 3004).

A mail ballot by the members of the Judicial Commission is scheduled. If there are objections to the proposal, decision will be deferred until the August 1962 meeting of the Commission at Montreal at the time of the International Microbiological Congress.

References

Pridham, T. G. and A. J. Lyons, Jr. 1961. *Streptomyces albus* (Rossi Doria) Waksman et Henrici: Taxonomic study of strains labelled *Streptomyces albus*. Jour. Bact. 81:431-441.

Lyons, A. J. Jr. and T. G. Pridham. 1962. Proposal to designate Strain ATCC 3004 (IMRU 3004) as the neotype strain of *Streptomyces albus* (Rossi Doria) Waksman and Henrici. Jour. Bact. 83:370-380.

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[1] Permission to reproduce portions of the International Bulletin of Bacteriological Nomenclature and Taxonomy pertinent to the *Streptomyces albus* neotype problem was granted by the Chairman of the Editorial Board, Dean R. E. Buchanan, Iowa State University, Ames, Iowa, USA.

Vol. 12, No. 3, pp. 123-126, July 15, 1962.

Proposal to Designate Strain ATCC 3004 (IMRU 3004) as the Neotype Strain of Streptomyces albus (Rossi Doria) Waksman and Henrici.

Thomas G. Pridham[2] and Allister J. Lyons, Jr.

SUMMARY

Strain ATCC 3004 (American Type Culture Collection; IMRU 3004 Institute of Microbiology, Rutgers University) is proposed as the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici 1943. Streptomyces albus is by original designation the type species of the genus Streptomyces Waksman and Henrici 1943.

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In view of the facts listed below we propose that the Judicial Commission of the International Committee on Bacteriological Nomenclature of the International Association of Microbiological Societies render an Opinion designating strain ATCC (American Type Culture Collection) 3004, (IMRU (Institute of Microbiology, Rutgers University) 3004) as the neotype strain of the species Streptomyces albus (Rossi Doria) Waksman and Henrici (basionym Streptotrix alba [sic] Rossi Doria 1891). This strain would then serve as the neotype strain of the type species of the genus Streptomyces Waksman and Henrici 1943.

1. In a recent survey of 26 specialists in the field of actinomycete taxonomy, 14 favored the International Code of Nomenclature of Bacteria and Viruses as the basis for nomenclature of the Actinomycetales.

2. Descendants of the holotype culture of Streptotrix [sic] alba Rossi Doria 1891 are no longer extant.

3. No type strain or neotype strain has been officially designated.

4. R. Hütter (1961) has proposed that strain ATCC 618 be designated as the neotype strain. However, insofar as can be determined, the history of this strain can be traced back to Krainsky in 1914. The history of strain ATCC 3004 (IMRU 3004) can be traced back to Berestnev in the 1890's.

5. S. A. Waksman (1961, p. 172) designates strain IMRU 3005 as the "type culture." The characteristics of this strain do not conform to those cited by Waksman and Henrici (1943). The strain exhibits characteristics attributed to Streptomyces griseus.

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Member, Subcommittee on Taxonomy of Actinomycetes of the Committee on Taxonomy of the American Society for Microbiology; and the Subcommittee on Taxonomy of the Actinomycetes of the International Committee on Bacteriological Nomenclature.

6. The characteristics of Rossi Doria's isolates suggest that the organism he studied was, in fact, an isolate of what is now recognized as Streptomyces griseus (Krainsky) Waksman and Henrici.

7. The descriptive material in the Rossi Doria publication leaves much question with regard to the identity of the organism with which he was working; so much so that Waksman and Henrici in 1943 proposed a concept for S. albus that bears little or no resemblance to the concept proposed by Rossi Doria.

8. Since about 1919, the characteristics for S. griseus have been presented in such fashion that there is little difficulty in assigning a given isolate to this species based on the characteristics required. The species, because of the work of Waksman and his collaborators, is well established in actinomycete taxonomy. Any effort to relate these strains to S. albus, based on the incomplete Rossi Doria concept (as measured by today's criteria in actinomycete taxonomy), would cause great confusion and result in greater chaos than is now present in actinomycete taxonomy.

9. Based on the 1943 Waksman and Henrici concept for S. albus there is little difficulty for specialists in the field to identify a given isolate with this species.

10. Strain ATCC 3004 (IMRU 3004) fulfils the requirements for classification in the species Streptomyces albus as the species is currently defined.

11. Strain ATCC 3004 (IMRU 3004) apparently is a viable descendant of the oldest known streptomycete isolate bearing the label "albus."

12. In support of the above statement we would like to call to the attention of the Commission the appended references. With regard to the references cited, attention should be called to several points.

    1. The reason for the change of proposed strain number from ATCC 618 to ATCC 3004 (IMRU 3004) is explained in the footnote on page 370 of the Lyons and Pridham article (1962).

    2. Information given on page 369, paragraph 2, of the Hütter article is incorrect. Neither of the two strains mentioned would be classified as strains of S. griseus. Strain NRRL B-1685 is, in fact, our designation for strain ATCC 618 which the author proposed as the neotype strain of S. albus. Strain ATCC 3004 is the strain characterized in our 1962 publication.

    3. Strain IMRU 3005, designated as the type culture of S. albus on page 172 of the Waksman volume 2 (1962), has characteristics that conform with those of S. griseus, based on our study of a culture of the strain obtained directly from Waksman.

References

Hütter, R. 1961. Zur Systematik der Actinomyceten 5. Die Art Streptomyces albus (Rossi Doria emend. Krainsky) Waksman and Henrici 1943. Archiv für Mikrobiologie 38:367-383.

Lyons, A. J. Jr., and T. G. Pridham. 1961. Proposal to designate strain ATCC 618 as the neotype strain of Streptomyces albus (Rossi Doria) Waksman et Henrici. Abstract in: Bacteriol. Proc. 1961:74.

_____ and _____. 1962. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman et Henrici. Jour. Bact. 83:370-380.

Pridham, T. G. and A. J. Lyons, Jr. 1960. Streptomyces albus (Rossi Doria emend. Krainsky) Waksman et Henrici: Taxonomic study of 42 strains labelled Streptomyces albus. Abstract in: Bacteriol. Proc. 1960:80.

_____ and _____. 1961. Streptomyces albus (Rossi Doria) Waksman et Henrici: Taxonomic study of strains labelled Streptomyces albus. Jour. Bact. 81:431-441.

Waksman, S. A. 1961. The Actinomycetes--Classification, Identification and Description of Genera and Species. The Williams and Wilkins Co., Baltimore. 363 pp.

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Vol. 13, No. 1, pp. 23-30, January 15, 1963.

DETAILED MINUTES CONCERNING ACTIONS TAKEN ON OPINIONS

During the Meetings of the Judicial Commission of the International Committee on Bacteriological Nomenclature at the VIII International Microbiological Congress in Montreal, 1962, W. A. Clark and H. P. R. Seeliger, Joint Permanent Secretaries.

Minute 67. Request for designation of a neotype strain for Streptomyces albus (Rossi Doria) Waksman and Henrici. Lyons and Pridham (1962) proposed that the strain IMRU No. 3004 (Institute of Microbiology of Rutgers University) and carried at the American Type Culture Collection as ATCC No. 3004, be designated as the type strain (culture) of Streptomyces albus (Rossi Doria) Waksman and Henrici (basionym Streptotrix [sic] alba Rossi Doria 1891). The Commission recognized the excellent documentation and presentation but concluded that the opinion of Dr. Gottlieb as to the sentiment of the International Subcommittee on the Actinomycetales, of which he is chairman, should be asked. The Chairman of the Judicial Commission was requested to take up the proposal with Dr. Gottlieb and transmit his suggestions to the members of the Judicial Commission. Final decision is to be by letter ballot.

References

Pridham, T. G. and A. J. Lyons, Jr. 1960. Streptomyces albus (Rossi Doria emend. Krainsky) Waksman et Henrici: Taxonomic study of 42 strains labelled Streptomyces albus. (Abstr.) Bacteriol. Proc. 1960:80.

_____ and _____. 1961. Streptomyces albus (Rossi Doria) Waksman et Henrici: Taxonomic study of strains labelled Streptomyces albus. Jour. Bact. 81:431-441.

Waksman, S. A. 1961. The Actinomycetes--Classification, Identification and Description of Genera and Species. The Williams and Wilkins Co., Baltimore, 363 pp.

Hütter, R. 1961. Zur Systematik der Actinomyceten 5. Die Art Streptomyces albus (Rossi Doria emend. Krainsky) Waksman and Henrici 1943. Archiv für Mikrobiologie 38:367-383.

Lyons, A. J. Jr., and T. G. Pridham. 1961. Proposal to designate strain ATCC 618 as the neotype strain of Streptomyces albus (Rossi Doria) Waksman et Henrici. (Abstr.) Bacteriol. Proc. 1961:74.

_____ and _____. 1962. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman et Henrici. Jour. Bact. 83:370-380.

Pridham, T. G. and A. J. Lyons, Jr. 1962. Proposal to designate strain 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici. Intl. Bull. Bact. Nomen. Taxon. 12:123-126.

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Vol. 13, No. 2, pp. 123-124, April 15, 1963.

OPINION 29

Designation of strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici.

Lyons and Pridham (1962) proposed that the American Type Culture Collection strain ATCC 3004 (Institute of Microbiology of Rutgers University, IMRU 3004) be designated as the neotype strain (culture) of Streptomyces albus (Rossi Doria) Waksman and Henrici (basionym Streptotrix [sic] alba Rossi Doria 1891). The Judicial Commission, at its meetings in Montreal in August 1962, deferred formal action until the proposal had been submitted to the Subcommittee on the Taxonomy of the Actinomycetales of the International Committee on Bacteriological Nomenclature. The proposal was transmitted to Dr. David Gottlieb, Chairman of the Subcommittee. In a letter to the Chairman of the Judicial Commission, Dr. Gottlieb replied:

> "The proposal has received enough publicity so that any objections could have been raised. Since they were not, I see no reason for withholding the designation of the strain IMRU No. 3004 as the neotype."

On November 9, 1962 the Chairman circulated the proposal among the members of the Judicial Commission. The Commissioners replied by letter ballot and, by 12 affirmative ballots (with 2 abstentions), approved the following Opinion:

Opinion 29. The strain labeled 3004 in the American Type Culture Collection, Washington, D. C. and also known as IMRU 3004 (Institute of Microbiology of Rutgers University) is designated as the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici 1943.

References

Pridham, T. G. and A. J. Lyons, Jr. 1960. Streptomyces albus (Rossi Doria emend. Krainsky) Waksman et Henrici: Taxonomic study of 42 strains labelled Streptomyces albus. (Abstr.) Bact. Proc. 1960:80.

_____ and _____. 1961. Streptomyces albus (Rossi Doria) Waksman et Henrici: Taxonomic study of strains labelled Streptomyces albus. Jour. Bact. 81:431-441.

Waksman, S. A. 1961. The Actinomycetes--Classification, Identification and Description of Genera and Species. The Williams and Wilkins Co., Baltimore. 363 pp.

Hütter, R. 1961. Zur Systematik der Actinomyceten 5. Die Art Streptomyces albus (Rossi Doria emend. Krainsky) Waksman and Henrici 1943. Archiv für Mikrobiologie 38:367-383.

Lyons, A. J. Jr., and T. G. Pridham. 1961. Proposal to designate strain ATCC 618 as the neotype strain of Streptomyces albus (Rossi Doria) Waksman et Henrici (Abstr.) Bact. Proc. 1961:74.

_____ and _____. 1962. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman et Henrici. Jour. Bact. 83:370-380.

Editorial Board. 1962. Proposal for the recognition of the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici 1943, p. 339. Intl. Bull. Bact. Nomen. Taxon. 12:65.

Pridham, T. G. and A. J. Lyons, Jr. 1962. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi Doria) Waksman and Henrici. Intl. Bull. Bact. Nomen. Taxon. 12:123-126.

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Northern Utilization Research and Development Division
Peoria, Illinois

STARCH FROM CEREAL GRAINS

A Short Method for Laboratory Extraction

Preparing samples of starch from corn and other cereal grains often is a problem to the researcher. Many times only very small quantities of grain are available, and in other cases only minute quantities of starch are needed. A short method has been devised for recovering starch from cereals, which can be carried out in most chemical laboratories. Only a minimum amount of readily available equipment is required, and the various operations are relatively simple and straightforward. This method is patterned after a bench-scale procedure for wet milling grains (1,2).

The corn is steeped, in an appropriate vessel, in an amount of water equal to twice the weight of the corn. The water may contain sulfur dioxide (as high as 0.25 gram/100 milliliters of steep water), the steeping agent used in industrial practice; or if a native starch is desired, the steeping agent should be distilled water, containing a small quantity of a preservative, such as Roccal.[1] Steeping of corn is generally carried out for 48 hours at a temperature of 125° F. (52° C.).

After the corn is steeped, the water is drained off, and the corn is ground in an appropriate mill, such as a drug mill, coffee mill, or meat grinder. Several passes through the mill may be necessary to achieve the proper grind.

Ground fibers and germ may now be separated from the mill starch (starch and gluten) by screening the mass on a 200-mesh stainless screen or a No. 17XXX silk bolting cloth. The mill starch is worked free from the fibers and germ on the screen by hand and collected. The fibers and germs are washed several times by resuspending them in water and then rescreening, collecting the washings, and combining them with the mill starch.

The mill starch is separated into starch and gluten by centrifugation in an International centrifuge. The crude starch is placed in 250-milliliter bottles, and spun at 2,000 r.p.m. for 15 minutes. The water layer is removed, and the dark layer of gluten is carefully scraped from the starch surface. The starch layer can be resuspended in water and again treated as above, thereby purifying the starch. The washing step can be repeated as many times as thought necessary.

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[1] Mention of trade products or firm names does not imply that they are endorsed or recommended by the Department of Agriculture over other similar products or firms not mentioned.

The purified starch can be dried in a hot-air drying oven. Temperature of drying should not exceed 125° F. (52° C.).

This short method should result in the recovery of a good grade of starch from corn. It is also applicable for extracting starch from other grains. Operating variables may be altered to suit individual preference.

Selected References

1. Anderson, Roy A., "Wet-Milling Properties of Grains: Bench-Scale Study," CEREAL SCI. TODAY 8: 191-195, 221 (1963).

2. Zipf, R. L., Anderson, R. A., and Slotter, R. L., "Wet Milling of Grain Sorghum," CEREAL CHEM. 27: 463-476 (1950).

Reprints may be obtained for reference 1 from the Northern Utilization Research and Development Division, 1815 North University Street, Peoria, Illinois 61604.